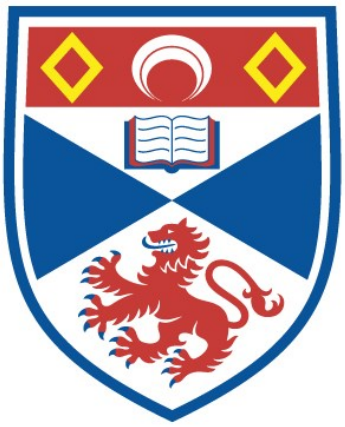
THE ORIGIN AND MAINTENANCE OF A NEW 'SENECIO' HYBRID IN YORK, ENGLAND

Andrew John Lowe

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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The Origin and Maintenance of a New *Senecio* Hybrid in York, England.

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A thesis submitted to the
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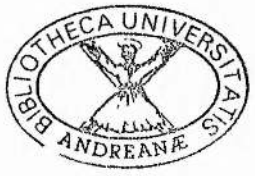

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ABSTRACT

The principal aim of the research presented in this thesis was to use a broad range of analytical techniques to investigate the origin and maintenance of a newly arisen *Senecio* hybrid, referred to as York radiate groundsel, in York, England.

The results of morphological and molecular analyses, showed that York radiate groundsel is a hybrid product between *Senecio vulgaris* var. *vulgaris* L. and *S. squalidus* L, and is distinct from the stabilized introgressant, *S. vulgaris* var. *hibernicus* Syme. These findings also indicated that York radiate groundsel is unlikely to be a first generation hybrid, but has probably undergone limited backcrossing to *S. vulgaris* with current populations of the plant derived from a single origin.

Tetraploid hybrid progeny between *S. squalidus* and *S. vulgaris* were synthesized artificially and some were partially fertile and similar in morphology to York radiate groundsel. It was argued that unreduced *S. squalidus* gametes are moer likely to have been involved in the origin of the new hybrid.

Postzygotic breeding barriers were shown to exist between York radiate groundsel and both of its parents. Backcrosses to *S. squalidus* were triploid and highly sterile, while backcrosses to *S. vulgaris* were tetraploid, but exhibited a significant reduction in seed and pollen fertility in the subsequent generation. Although flowering time of York radiate groundsel and *S. vulgaris* differed substantially, an examination of capitula number in relation to flowering time, revealed that the flowering time difference was probably not selected as a prezygotic isolating mechanism. The results of a field experiment that measured outcrossing rates, suggested that York radiate groundsel may be more attractive to pollinators than either variety of *S. vulgaris*, which would in turn, lead to its effective ethological reproductive isolation from these taxa. It was also shown that characters that could promote pollinator attraction in the new hybrid, have probably been inherited from *S. squalidus* and have remained associated with the ray floret locus in York radiate groundsel due to gene linkage.

The effects of inbreeding on fitness were found to be negligible in York radiate groundsel and this, together with the fact that it exhibited some novel ecological characteristics, is discussed in regard to its continued maintenance in the wild.

The distinctive morphological and molecular phenotype of York radiate groundsel and the fact that it has achieved a level of reproductive isolation from both of its parents, should favour its recognition as a new species.

DECLARATION

I, Andrew John Lowe, hereby certify that this thesis, which is approximately 63,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Andrew Lowe
September 1996.

STATEMENT

I was admitted as a research student in October, 1992, and as a candidate for the degree of Ph.D. in October, 1993; the higher study for which this is a record was carried out in the University of St. Andrews between 1992 and 1996.

Andrew Lowe
September 1996.

CERTIFICATE

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Ph.D. in the University of St. Andrews and that the candidate is qualified to submit this thesis in application for that degree

R.J. Abbott
September 1996.

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The financial assistance of a Natural Environment Research Council research studentship is gratefully acknowledged.

Paul Klee, *Senecio* (1922)

A careful examination of the nature of nature.

CONTENTS

Chapter 1.
General introduction

To understand the processes that generate species it is first necessary to classify the great diversity of organisms. In an attempt to produce order from a world of myths and uncertainty, the ancient Greeks devised various systems of classification for nature. For example, Plinius developed a threefold system for animals living on land, in water and in the air (Dobzansky *et al.*, 1977), while Aristotle distinguished the main classes of creature based on multiple differentiae (Russell, 1961). The single most important contribution towards classification was made by Linnaeus (1707-78), who devised a hierarchy of systematic categories for plants and animals mainly based on floral and body structure. His binomial nomenclature and view that every organism must belong to the lowest taxonomic entity, the species, remain largely valid today. He also assigned labels (family, genus and species) to nearly all the species of flora and fauna known to the western world at that time, most of which have been maintained. Initially, Linnaeus held a very rigid view on species since he believed them all to be well defined, unchanging entities created in a single act by God. However, later in his life his fixed views on species broadened as he became increasingly aware of instances of hybridization (Dobzansky, *et al.*, 1977). Darwin and Lamark had a much more fluid idea of species and believed they could change or split over time (Darwin, 1859). To them this idea was strongly reinforced by the difficulties encountered in classifying many animal and plant groups.

Concepts of species

A workable concept of a species was necessary for classification and, initially, differences in morphological features became the basis for a taxonomic species concept. However, it was Dobzansky's (1937) observation that the process responsible for species formation is the development of pre- and/or postzygotic reproductive isolating barriers that lead to the biological species concept (BSC). Mayr (1942) defined species as 'groups of [actually or potentially] interbreeding natural populations which are reproductively isolated from other such groups'. Most criticisms of this concept are aimed at its taxonomic application, as large numbers of sibling or semi-species, that are sexually isolated, but difficult to differentiate, are classified as separate species under this definition (Grant, 1957). Moreover, asexual species (including apomicts) are not included in this definition. Sokal and Crovello (1970) observed that if two species hybridized in areas of sympatry and formed interfertile backcrosses, then strict application of the BSC would imply they were one species. To circumvent that problem they favoured a numerical taxonomic definition

that emphasised the phenetic differences between species. However, in general the BSC is not strictly applied in cases of limited hybridization and a low level of interbreeding is usually tolerated (King, 1993).

A number of other species concepts have been proposed based on the foreseen shortcomings of the BSC. Paterson (1985) put forward the recognition species concept in which species are those which share a common mate recognition system. However, this definition implies that all species capable of producing hybrid progeny, whether fertile or sterile, are one species. In the cohesive species concept (CSC), Templeton (1989) altered the BSC to stress the importance of mating mechanisms which facilitate reproduction and not the negative aspects of isolation, which can not be selected for. The latter two concepts are extremely similar and King (1993) questioned whether the CSC makes a significant enough contribution so as to be recognized separately from the BSC.

Simpson (1961) put forward the evolutionary species concept (ESC) to allow for recognition of extinct species. This was based on the idea that the evolutionary lineage of a species included a number of extinct species and, therefore, the lineage had been transformed through several species. The ESC was altered by Wiley (1978) to define species as a single lineage that maintains its identity from other lineages, and is useful in a paleontological context. The ecological species concept (van Valen, 1976) recognized the importance of ecological niches and their influence on an individual's development and genotypic selection. However, this definition has several shortcomings, it implies that two different species cannot occupy the same niche, and that a species forced to extinction through competition could not have been a real species (Wiley, 1978). The phylogenetic species concept (Cracraft, 1983), emphasized the distinctive taxonomic differences (mainly morphological, but also biochemical, physiological and behavioural) between species (as it is these characters that prevent inter-breeding with other species), and individuals of a species are those within which there is a parental pattern of ancestry and decent. However, this concept fails to establish at what level of character differentiation a new species should be recognized (as was the case for the numerical taxonomic approach of Sokal and Crovello, 1970). For example, DNA sequencing or 'fingerprinting' of certain genomic regions could differentiate every individual of a population, but they should not be classified as different species (Avise, 1994).

In summary, the biological species concept is still the most widely used and applied by evolutionary biologists, and although other concepts make valid contributions to the

understanding of the idea of a species, they are rather specialized and not generally superior to the BSC (King, 1993).

Speciation

As regards the genetic basis of speciation, de Vries (1915) believed that differences between species could be expressed in units of single gene mutations. However, Morgan (1932) pointed out that even closely related species may differ from each other for many gene changes. It was the accumulation of beneficial genetic changes that Wright (1932) envisioned as the process which drove speciation through an adaptive landscape, with mutations of small effect allowing fine tuning to the summit of adaptive peaks (Fisher, 1930); and those of large effect (Templeton, 1981) or a population crash followed by genetic drift (shifting balance, Wright, 1932) allowing shifts between peaks, possibly to ones with a higher adaptive summit.

The genetic basis of speciation is, however, difficult to discern as the events that have been involved are usually obscured by time (Harrison, 1991). Indeed Lewontin and Birch (1966) observed that; 'it is a fundamental difficulty of an historical science like evolution that one can never establish the cause of a past event. It is only possible to show that certain causes are plausible or at most likely, but because each species is a unique historical event we cannot say for certain what its genetic history was'. Several major theories have been subtended to explain the origin of species, and can be split into gradual (involving polygenic systems) and sudden (involving major genes) speciation events. Avise (1994) observes that careful application of methods studying biochemical and genetic variation may allow an insight into historical processes that lead to speciation, although recent events are more likely to be correctly identified.

Gradual speciation

The classic idea of speciation refers to populations which have been spatially isolated (King, 1993), and which become genetically differentiated due to the combined effects of mutation, genetic drift, selection and migration (Avise, 1994). If after a period of isolation, two populations come into secondary contact, speciation will be complete if the gene differences are enough to cause reproductive isolation. This process has been termed gradual allopatric speciation.

Many crossing studies have been undertaken to examine the occurrence of reproductive barriers between closely related plant taxa e.g. between subspecies of *Layia glandulosa* (Clausen, 1951) and within the *Mimulus guttatus* complex (Vickery, 1964). The results broadly show that taxa classified as more closely related had no or

weaker breeding barriers between them than did taxa that had been classified as more distantly related. In *Drosophila*, reproductive isolation of species was positively correlated with the degree of genetic divergence measured by isozyme diversity (Coyne and Orr, 1989). However, the nature of the isolating mechanisms was not specified and premating isolation was not included. The relevance of genetic divergence of certain, supposedly, neutral gene markers to reproductive isolation, is also open to debate (King, 1993).

The colonization of oceanic islands and ensuing adaptive radiation into available niches is thought responsible for the evolution of many island endemics (Carlquist, 1995). For example, approximately 800 of 2000 *Drosophila* species can be found on Hawaii (Avise, 1994). The study of speciation on oceanic islands has been viewed as an ideal situation to study the processes of evolution (Carlquist, 1995), although it is debatable whether it represents only a special case of speciation (King, 1993). Several theories of speciation on oceanic islands have been modelled including the founder flush and transilience models (Carson and Templeton, 1984; Giddings, Kaneshiro and Anderson , 1989), although these are not universally accepted (Barton, 1989).

One of the most important concepts of gradual speciation was initially developed by A. R. Wallace and reiterated by Dobzansky (1951), who postulated that reproductive isolation could develop as a by product of genetic divergence or be reinforced by selection against unfit hybrid progeny (Grant, 1966). Howard (1993) refined the definition of reinforcement, as the evolution of prezygotic isolating barriers in zones of hybridization as a response against hybridization. The highly developed sense organs of animals were postulated as the main vectors of such a speciation process either through mate selection systems in animals (see Howard, 1993) or by pollinator choice in plants (Grant, 1949). Grant (1949) noted that the flower constancy of bees could form the basis of a speciation process and colour divergence in sympatric *Phlox* populations has been linked with pollinator mediated prezygotic isolation (Levin and Kerster, 1967). Hewitt (1988) has noted that there are theoretical problems with the reinforcement model, while Butlin (1989) has argued that there are no unequivocal natural examples of such a process. Butlin and Ritchie (1994) observed that behaviour patterns in mammals that do contribute to speciation evolve as a result of processes occurring within species, and the resulting barriers to gene exchange are incidental consequences, rather than functions of the characters involved.

In some cases (e.g. *Pinus muricata*, Millar, 1983), the causes of divergence in flowering time have been assigned to reinforcement (in Butlin, 1989), although

selection probably acts to maximize ecological fitness rather than minimize unfit hybrid production, and has been termed micro-allopatric phenological separation. The influence of diversifying natural selection on resource utilization has been shown to cause host shifts and associated speciation events in a number of insect species (Tauber and Tauber, 1989), but is mainly confined to phytophagous and zoophagous parasites and parasitoids (Bush, 1975). In recognition of the isolation accompanying a host shift the process has been viewed as micro-allopatric speciation.

Although speciation by reinforcement, as Dobzansky (1951) envisioned it, has been difficult to establish in natural systems (Butlin, 1989; Butlin and Ritchie, 1994), reproductive character displacement (Brown and Wilson, 1956) has been shown to occur. Butlin (1989) defines reproductive character displacement as the selection for character divergence in the absence of gene exchange. The diversity of calls exhibited by species of Hawaiian crickets, *Laupala* (Otte, 1989), is most probably due to this process (Butlin and Ritchie, 1994).

Sudden speciation

Several processes are thought to be responsible for sudden speciation events. Lewis (1966) considered that saltational speciation would most likely occur when one or a few individuals became isolated in a new habitat from an original population. Multiple simultaneous chromosome rearrangements might be generated by enforced inbreeding within the daughter population and 'catastrophic' selection could favour a few individuals. Two predictions come from this hypothesis; first in groups undergoing active speciation, imperfect barriers of segregational hybrid sterility will exist in some hybrids between populations of the same species (as has been observed in *Clarkia* by Lewis 1966). Second, certain chromosome patterns in different populations of a species should exhibit a regular ecological distribution (eg in *Trillium*, Stebbins, 1971). Good examples of saltational speciation concern the origin of *Coreopsis nuecensoides* from *C. nuecensis* (Crawford and Smith, 1982), *Stephanomeria malheurensis* from *S. exigua* ssp. *coronaria* (Brauner and Gottlieb, 1987) and in *Clarkia* (Lewis, 1966).

Self-fertilization favours the establishment of chromosomal rearrangements and contributes towards the reproductive isolation in saltational speciation. However, the evolution of selfing is itself one of the most prominent evolutionary pathways leading to species formation in herbaceous plants (Stebbins, 1957; Barrett, 1989). Shifts to autogamy have been recorded in sibling species and self-incompatible groups (*Holocarpa* and *Layia*, Clausen, 1951). Selfing is at an advantage in low density populations and is favoured in pioneer environments or populations that frequently

experience bottlenecks (Barrett, 1989). A frequent observation is that selfers tend to occur at the geographic/ecological margins of the outcrossing progenitors (Jain, 1976).

Role of hybridization in speciation

Species definitions and speciation processes imply that there is a lack of gene flow between populations that are diverging, but hybridization between two well established species may also lead to speciation. Hybridization is now recognized as a major route in the evolution of species (Anderson, 1949, 1953; Stebbins, 1959; Stace, 1975; Levin, 1979; Grant, 1981), although in the past it has been considered of minor importance (Wagner, 1970; Mayr, 1963). Hybridization is much more common in plants and lower animals (invertebrates, fish and amphibians) than in higher animal taxa (Mayr, 1963; Stace, 1975, Avise, 1994). The relatively simple structure of plants and lower animals is the most probable reason why these groups are tolerant of the developmental problems associated with hybridization (Dobzansky *et al.*, 1977).

Hybridization between species can result in introgression, the establishment of hybrid zones or swarms, and the origin of new species at the homoploid or polyploid level. Backcrossing of hybrids to the parental species and associated transfer of genetic material is known as introgression (Anderson and Hubricht, 1938; Anderson, 1949). In the past, many cases of introgression have been inferred but not investigated in detail (Avise, 1994). However, the process is undoubtedly common and in a recent review, Rieseberg and Wendel (1993) listed 165 plant examples, many of which are supported by molecular evidence. Notable examples of introgression, that have been well documented, have been reported in *Helianthus* (Heiser, 1951; Rieseberg, Beckstrom-Sternberg and Doan, 1990), *Senecio* (Abbott, Ashton and Forbes, 1992), *Clarkia* (Bloom, 1976) and sunfish (Hubbs, 1955). Introgression of genes causing gene pool enrichment may help colonization of new habitats (Abbott, 1992), for example, introgression of genes from *Dacus neohumeralis* into *D. tryoni* increased heat tolerance (Lewontin and Birch, 1966). The process can also lead to the origin of stabilized introgressants that may be recognized at a low taxonomic level e.g. *Helianthus annuus* ssp. *texanus* (Heiser, 1951; Rieseberg, Beckstrom-Sternberg and Doan, 1990) and *Senecio vulgaris* var. *hibernicus* (Abbott, Ashton and Forbes, 1992).

Localized hybridization of two formerly allopatric or parapatric taxa in secondary contact may form linear (Hewitt, 1989) or mosaic (Harrison and Rand, 1989) hybrid zones. If hybrid zones are not ephemeral, then two models favour their persistence. The bounded hybrid superiority hypothesis, predicts that hybrid fitness will be superior

to parental populations in certain habitats (Moore, 1977). In contrast, the dynamic equilibrium model, predicts that hybrids will exhibit lower fitness than parental taxa regardless of habitat, but the hybrid zone is maintained by recurrent hybrid formation (Barton and Hewitt, 1985). A recent review by Arnold and Hodges (1995) found that many hybrids were not uniformly unfit, a finding that favours the bounded hybrid superiority hypothesis. Anderson (1949) noted that human habitat disturbance often produced a hybridized habitat in which a range of hybrid progeny could survive. This process was probably responsible for the production of many hybrid swarms between *Iris fulva* and *I. hexagona* var. *giganticoerulea* in Louisiana (Anderson, 1948, 1953).

Persistence of hybrid zones may allow the introgression of genes across species barriers. For example, genes from *Mus domesticus* have introgressed into *M. musculus* across a linear hybrid zone that splits Europe, whereas, the reciprocal transfer of genes has not occurred (Hunt and Selander, 1973). There has also been transfer of *M. domesticus* mitochondrial DNA (mtDNA) into Scandinavian populations of *M. musculus* without associated transfer of nuclear DNA markers (Gyllensten and Wilson, 1987). There are several special circumstances that may account for differential introgression of plastid and nuclear genes. In animal species which possess sex chromosomes of unequal size, the phenomenon where the heterogametic sex is absent, rare or sterile in F_1 offspring between two species is known as Haldane's rule (1922, in Avise, 1994). This process has been observed in a number of taxa (Coyne and Orr, 1989; Avise, 1994), and under certain circumstances may facilitate the transfer of mtDNA across species boundaries (Avise, 1994). Chloroplast DNA (cpDNA) capture is also commonly observed in some plant species e.g. in *Gossypium* (Wendel, Stewart and Rettig, 1991) and in *Quercus* (Ferris *et al.*, 1993). One reason for such cyto-nuclear disequilibrium in plants may be the differential gamete exchange due to the dispersal properties of pollen and seed (Arnold, 1992; Ennos, 1994).

Following hybridization, chromosome segments that distinguish the parental species may recombine in hybrid progeny producing new recombinant types that are fertile *inter se,* but at least partially sterile with both parents. This process has been termed 'recombination speciation' by Grant (1981), and has been shown to occur experimentally in crosses between *Gilia malior* and *G. modocensis* (1966). Templeton (1981) suggested that hybridization may induce mutator activity, hybrid dysgenesis, resulting in high levels of chromosome breakage and genic divergence, for which there is some evidence (Barton, Halliday and Hewitt, 1983). Recent studies also suggest that the process of chromosome recombination and repatterning may be

restrained by fertility, and some chromosomal rearrangements may be non-random (Rieseberg, 1995; Rieseberg, Fossen and Desrochers, 1995; Rieseberg *et al.*, 1996). Examples of species believed to have originated via recombination speciation include *Stephanomeria diegensis* (Gallez and Gottlieb, 1982), *Iris nelsonii* (Arnold, Bennett and Zimmer, 1990; Arnold, Buckner and Robinson, 1991; Arnold, 1993), *Helianthus paradoxus* (Rieseberg, Beckstrom-Sternberg and Doan, 1990), *Plethodon teyahalee* (Highton, Maha and Maxson, 1989) and *Gila seminuda* (DeMarais *et al.*, 1992).

The process of hybridization may also affect normal fertilization processes causing prezygotic reproductive isolation. Hybridization is known to affect morphological character coherence (Rieseberg and Ellstrand, 1993; Rieseberg, 1995) and may disrupt floral structure in hybrid progeny causing reproductive isolation from parental taxa and sudden speciation (Grant, 1949) e.g. in *Penstemon* (Straw, 1955).

Importance of polyploidy in speciation

Sterile hybrids may be propagated by asexual modes of reproduction, and Ellstrand, Whitkus and Rieseberg (1996) have noted that most plant groups in which hybridization occurred were principally outcrossing perennials with reproductive modes that stabilized hybridity, such as agamospermy, vegetative spread or permanent odd ploidy. For example, clonal propagation is responsible for the spread of *Spartina* x *townsendii* (Gray, Marshall and Raybould, 1991) and in *Potamogeton* (Hollingsworth, Preston and Gornall, 1995, 1996), and apomixis has allowed propagation in *Rosa* and a proliferation of *Taraxacum* 'micro species' (Stace, 1991). However, in evolutionary terms, asexual modes of reproduction are viewed as 'dead ends'. Hybrid sterility, however, may be overcome by polyploidy.

Polyploidy is the situation when cells of an organism contain multiples of the diploid chromosome set, and is usually caused by the fusion of unreduced gametes (Harlan and de Wet, 1975; Bretagnolle and Thompson, 1995). By taking a basic chromosome number (x) greater than 13 to indicate polyploidy, Grant (1981) estimated that 47% of angiosperms were of polyploid origin. Lewis (1980) argued that chromosome numbers of x=9, 10 and 11 were also probably due to polyploidy and he obtained an estimate of 70-80% for angiosperms. The proportion of polyploids is even higher in ferns, for which it has been estimated by Grant (1981) that about 95% were derived following polyploidy (x>13). A remarkable example of a high level of polyploidy is found in *Ophioglossum reticulatum*, which has 1260 chromosomes, making it an 84-ploid (x=15, Briggs and Walters, 1984). Polyploidy is found in lower animals (invertebrates, fish and amphibians, Avise, 1994) although it is not nearly as common

as in plants (Thompson and Lumaret, 1992) probably due to the disruption it causes to development (King 1993).

In general, polyploids are immediately reproductively isolated from their progenitor species due to chromosome mispairing and associated infertility in backcross progeny. Polyploids in which both chromosome sets are derived from a single species are referred to as autopolyploids, and those in which two or more species are involved are allopolyploids. The majority of chromosomes in allopolyploids usually form bivalents and segregate normally (Jenkins and Rees, 1991); however, autopolyploids tend to form multivalents and can experience reduced fertility due to segregational difficulties (King, 1993). There is some evidence that autopolyploid genomes extensively diploidize over time by processes of gross gene silencing, facilitating normal chromosome segregation (Soltis and Soltis, 1988; Soltis and Soltis, 1993). The difference between the two categories of polyploid may sometimes be difficult to distinguish. For example, if individuals from two very different populations of the same species, form an inter-population polyploid hybrid, it may form perfect bivalents due to the genetic differences between the populations, such a case is usually referred to as segmental allopolyploidy (Stebbins, 1947).

Polyploidy can contribute characters that are intrinsically important to the organism. For example, plants can have larger flowers, and seeds and firmer textured leaves, and these characters are products of what have been termed 'gigas' effects by de Vries and Gates (in Briggs and Walters, 1984). However, autopolyploids are not always more vigorous than their progenitors and many allopolyploids also exhibit novel morphological and molecular characters associated with their hybrid origin (Rieseberg and Ellstrand, 1993; Rieseberg, 1995; Soltis and Soltis, 1995; Song *et al.*, 1995). Allopolyploidy in plants is often accompanied by a shift in breeding system towards autogamy, whereas autopolyploids tend to remain outcrossing like their diploid progenitors (Barrett, 1989; Thompson and Lumaret, 1992). Autopolyploids are less frequent than allopolyploids in natural populations and have been viewed as maladaptive (in Soltis and Soltis, 1993). However, due to polysomic inheritance and frequent, multiple origins, autopolyploids exhibit enzyme multiplicity, increased heterozygosity, and increased allelic diversity relative to diploid progenitors (Soltis and Soltis, 1993). Autopolyploids usually arise on multiple occasions locally and within limited time periods (Soltis and Soltis, 1993), which allows outbreeding between polyploid individuals. The genetic attributes of autopolyploids provide strong arguments for their potential success in nature, although diversity needs to be maintained by outcrossing (Barrett, 1989). In contrast, heterozygosity in

allopolyploids results from gene multiplication (fixed heterozygosity) rather than allelic variation maintained by outcrossing (Soltis and Soltis, 1993) and so reproductive success can be assured by selfing without associated problems of inbreeding depression (Barrett, 1989). Notable products of polyploid evolution include four allopolyploid plant species known to have arisen this century *Senecio cambrensis* (Ashton and Abbott, 1992a), *Spartina anglica* (Gray, Marshall and Raybould, 1991), and *Tragopogon mirus* and *T. miscellus* (Ownbey, 1950; Roose and Gottlieb, 1976).

The study of a recently arisen fertile hybrid, York radiate groundsel

Hybridization is more prevalent in plants than animals and as such has a more crucial role in the evolution of new plant taxa. For example, in Stace's *New Flora of the British Isles* (1991), 715 of the 2834 plants that he treats fully are hybrids. The processes of speciation that have given rise to many hybrid plant taxa are relatively ancient and are now difficult to discern. The discovery of a recently evolved hybrid taxon offers the best opportunity to study evolution by hybridization. Such an opportunity has been presented by the discovery in 1979 of York radiate groundsel, a fertile tetraploid that is thought to be a recent hybrid product between the tetraploid inbreeder, *Senecio vulgaris*, and the diploid outcrosser, *S. squalidus* (Irwin and Abbott, 1992). Work in this thesis has used a broad range of analytical techniques to:

1. Examine the relationship between the York radiate groundsel and its putative parental taxa;
2. Resynthesize hybrids similar to York radiate groundsel from crosses between *S. vulgaris* and *S. squalidus*;
3. Examine the existence of pre- and postzygotic breeding barriers between York radiate groundsel and its parental taxa and;
4. Determine the effect of inbreeding on the continued survival of the new hybrid product.

Finally the taxonomic status of this new hybrid product is also briefly discussed.

Chapter 2.
Evidence that York radiate groundsel is a new and distinct hybrid taxon

Introduction

York radiate groundsel was first discovered in 1979 by R.J. Abbott and D.F. Marshall growing alongside a wall in a car park near York railway station. Irwin and Abbott (1992) demonstrated the intermediate nature of York radiate groundsel between its putative parental taxa *S. vulgaris* var. *vulgaris* and *S. squalidus* based on morphological and isozyme data, and showed that it was distinct from the stabilized introgressant *S. vulgaris* var. *hibernicus*. However, some workers have viewed York radiate groundsel as an extreme form of *S. vulgaris* var. *hibernicus* (Warren, 1987; Oxford, Crawford and Pernyes, 1996). Crisp (1972) found a single radiate groundsel individual in London that, from his descriptions, bore a close resemblance to extant York radiate groundsel individuals. Crisp suggested that taxonomic recognition could be given to such morphologically intermediate lines if they were persistent and self-maintaining. The populations of the plant in York comprise highly fertile, true breeding tetraploid individuals which have been expanding in number recently (e.g. in 1991 more than 250 individuals were recorded in York, Irwin and Abbott, 1992). The main objective of this chapter is to demonstrate further the intermediate and hybrid nature of York radiate groundsel between *S. vulgaris* var. *vulgaris* and *S. squalidus* and to show its distinctness from 'normal' *S. vulgaris* var. *hibernicus*, so as to justify its recognition as a distinct taxon.

Morphological characterization of hybrids

Traditionally, morphological characters have been the mainstay of classification and have been used extensively to demonstrate hybridization or introgression (Heiser, 1951; Stace, 1975, 1980). In Stace's *New Flora of the British Isles* (1991), most of the 715 hybrids recorded were proposed on the basis of morphological characteristics alone. However, morphological characters in hybrids are not always intermediate or of parental type, and hybrid characters may fall outside the range of variation observed in the parental species. In a recent review of morphological variation in 33 interspecific hybrids (Rieseberg and Ellstrand, 1993), 64% of first generation hybrids and 89% of later generation hybrids were shown to exhibit extreme or novel characters. Novel characters have been observed in the hybrid *Senecio cambrensis* where mean achene length, pollen grain diameter and number of pollen pores were significantly greater than the means recorded in the parental taxa, *S. vulgaris* and *S. squalidus* (Crisp, 1972; Taylor, 1984; Lowe and Abbott, 1996).

Hybrids have traditionally been identified using a hybrid index, where a number of characters are scored, with zero or low scores given for character magnitudes like one parent, and high scores for character magnitudes similar to the other parent. Truly intermediate hybrids should exhibit an intermediate hybrid index (Anderson, 1949). However, Anderson noted that this method gave no information on the actual character of hybrids. An improvement was to use scatter diagrams, where two quantitative characters were plotted on the x and y axes and several discrete characters could be represented as informative symbols (Anderson, 1949; Briggs and Walters, 1984). Hutchinson also promoted the use of polygonial graphs that allowed several characters to be plotted as multiple axes on one graph (in Briggs and Walters, 1984).

As increasingly powerful computer facilities have become more widely available, various multivariate statistical procedures have been used in the morphometric analysis of hybrids. These include, principal component analysis, principal coordinate analysis and canonical variate analysis (Manly, 1992). More recently, mathematical analyses of morphological variation have been developed with emphasis on the examination of biological shape and form. In particular, changes in specific homologous developmental structures or 'landmarks' (Bookstein *et al.*, 1985) have been used to demonstrate shape changes between closely related organisms. In these analyses, the outline of a structure is described by the coordinates of several important anatomical features and quantification of the relative distortion of these landmark positions between biological entities provides an informative description of shape change (Sneath, 1967; Gower 1971; Bookstein, 1978; Bookstein, 1989; Rohlf and Slice, 1990; Bookstein, 1991). Landmark analysis has been used to investigate hybridization between *Quercus rubra* and *Q. ellipsoidalis* with landmarks chosen to represent anatomical structures that differentiated the two species (Hill, 1980; Jensen, 1990). From 17 landmark coordinates, Jensen and co-workers (1993) derived nine linear measurements and three angular measurements of oak leaves that were analysed using principal component analysis (Manly, 1992; Somers, 1989). Rotational fit methods (Rohlf and Slice, 1990; Jensen 1990) were further used to analyse the landmark coordinate data directly. The two methods of analysis provided evidence that there had been a history of hybridization between the two oak species, mainly in the form of introgression from *Q. ellipsoidalis* into *Q. rubra*.

Although morphological characters can be very informative to questions of hybrid origin, problems can arise due to their limited number and the fact that shared characters may result from convergent evolution rather than shared derived ancestry.

Chromosome analysis of hybrids

Chromosome number provides crucial evidence on the ploidy level of a hybrid, whilst an examination of chromosome pairing at meiosis helps provide an understanding of the fertility exhibited by a hybrid. Previous studies have shown that the allohexaploid, *Senecio cambrensis*, exhibits either no irregular (Harland, 1954) or only slightly irregular (Crisp, 1972; Weir and Ingram, 1980) meiotic chromosomal pairing in wild and synthesized specimens, which, in turn, show high fertility. Conversely, the triploid hybrid between *S. vulgaris* (2n=4x=40) and *S. squalidus* (2n=2x=20), *S.* x *baxterii* (2n=3x=30), is nearly completely sterile and artificially synthesized triploids show great meiotic imbalance, usually forming 10 bivalents and 10 univalents (Ingram, 1977; Ingram, 1978; Ingram, Weir and Abbott, 1980).

The use of genomic *in situ* hybridization (GISH) to 'paint' chromosomes of suspected hybrids with DNA samples of the putative parents labelled with different fluorescent dyes, offers an exciting new tool for testing genome relationships between parent species and hybrids. In one study using this technique, the results strongly supported the assertion that *Milium montianum* was an allotetraploid with *M. vernale* donating the L-chromosome genome (Bennett, Kenton and Bennett, 1992).

Isozyme analysis of hybrids

Allozyme variation has been widely used to detect the hybrid or introgressive origins of several plant taxa, with hybrid derivatives normally exhibiting an additive parental phenotype (Gallez and Gottlieb, 1982; Crawford, 1985; Crawford and Ornduff, 1989). In *Senecio*, isozyme analysis confirmed the hybrid origin of *S. cambrensis* at two separate locations in North Wales and Edinburgh (Ashton and Abbott, 1992a) and also provided strong evidence for the introgressive origin of the radiate groundsel, *S. vulgaris* var. *hibernicus*, following introgression of *S. squalidus* genes into *S. vulgaris* var. *vulgaris* (Abbott, Ashton and Forbes, 1992). Similarly, it has been shown that the recently arisen allotetraploid, *Spartina anglica*, combines the diagnostic isozyme phenotypes of *S. alterniflora* and *S. maritima* and most probably arose by chromosome doubling of the sterile hybrid *S.* x *townsendii* (Raybould *et al.*, 1991; Gray, Marshall and Raybould, 1991). In addition, isozyme analysis has confirmed that the parents of *Tragopogon mirus* and *T. miscellus*, two recent products of allopolyploidy, are *T. dubius* and *T. porrifolius*, and *T. dubius* and *T. pratensis* respectively, and that there has been at least three separate origins of *T. mirus* (Roose and Gottlieb, 1976).

Although hybrid taxa can normally be detected by the possession of additive isozyme phenotypes, they may also be identified due to their intermediate position between

parental taxa in a dendrogram constructed from phenetic distance measures based on allozyme allele frequencies. In a dendrogram produced by the unweighted pair-group method of cluster analysis using arithmetic means (UPGMA) of allozyme phenotype frequencies, hybrids and introgressants between *Colochortus minimus* and *C. nudus* were placed in an intermediate position relative to the parental taxa (Ness, Soltis and Soltis, 1990). However, sometimes the resolution of hybrids in such trees may be ambiguous (Rieseberg and Ellstrand, 1993).

DNA analysis of hybrids

More recently, methods to examine DNA variation have been widely applied to the analysis of hybrids and hybrid derivatives. Of particular importance in this respect, have been studies that have analysed chloroplast and ribosomal DNA variation in plant hybrids and their putative parents. In an analysis of restriction fragment length polymorphism (RFLP) of nuclear rDNA most *Senecio cambrensis* individuals surveyed were found to exhibit an additive rDNA profile which combined the respective rDNA profiles of its putative parental taxa, *S. vulgaris* and *S. squalidus* (Harris and Ingram, 1992a; Harris and Ingram, 1992b). The same study, however, failed to provide any evidence of introgression of rDNA from *S. squalidus* into *S. vulgaris* var. *hibernicus*, as might have been expected given the known introgressant status of var. *hibernicus*. In a study of hybridization between *Iris* species in the south-east of the USA, Arnold, Bennett and Zimmer (1990) showed that individuals in allopatric populations of *I. fulva* and *I. hexagonia* possessed species specific rDNA RFLP profiles, although these differences were not found in areas where the two species were parapatric. In such areas, the distribution of rDNA RFLP markers among plants indicated that bi-directional back-crossing and introgression were common (Nason, Ellstrand and Arnold, 1992). Evidence from nuclear rDNA RFLP analysis (Arnold, 1993), also confirmed that the diploid species *Iris nelsonii* combined genetic markers found in allopatric populations of *I. fulva*, *I. hexagona* and *I. brevicaulis* and may, therefore, be considered to be a derivative of hybridization between these three species.

RFLP analysis of chloroplast DNA (cpDNA) variation has been used increasingly to ascertain the maternal parentage of hybrids (Rieseberg and Brunsfeld, 1992; Soltis, Soltis and Milligan, 1992). In the angiosperms, cpDNA is mainly maternally inherited (Harris and Ingram, 1991), whereas in gymnosperms paternal inheritance is more common (Stine and Keathley, 1990; Wagner *et al.*, 1991). RFLP analysis of cpDNA variation of the *Tragopogon* allotetraploids, *T. mirus* and *T. miscellus*, and their respective diploid parents, revealed that the maternal parent in three independent

origins of *T. mirus* was *T. porrifolius*; whereas both *T. dubius* and *T. pratensis* had acted as maternal parents in the recurrent origin of *T. miscellus* (Soltis and Soltis, 1989). RFLP analysis of cpDNA variation also confirmed that there have been at least two independent origins of *Senecio cambrensis* in the British Isles (Harris and Ingram, 1992a) and that *S. vulgaris* was the most likely maternal parent in both cases (Lowe and Abbott, 1996). Occasionally such molecular analysis can detect hybridization and introgression in the absence of morphological evidence; for example, RFLP analysis of cpDNA variation in *Rhododendron flammeum* and *R. canescens* revealed extensive localized introgression of the *R. canescens* chloroplast genome into individuals of *R. flammeum* without any morphological indication that hybridization had occurred (Kron, Gawen and Chase, 1993).

In the genus *Helianthus*, molecular analysis has been used extensively to study hybridization and the origin of hybrid taxa, and has proved particularly powerful in reconstructing evolutionary events when the analysis of cpDNA and rDNA are combined. For example diagnostic chloroplast and ribosomal DNA markers that distinguish the common sunflower, *Helianthus annuus*, from the cucumber-leaf sunflower, *H. debilis* ssp. *cucumerifolius*, were used to show that the cpDNA of *H. annuus* has been frequently transferred to *H. debilis* ssp. *cucumerifolius* without accompanying nuclear rDNA genes (Rieseberg, Choi and Ham, 1991). In another study, analysis of cpDNA, rDNA and isozyme variation effectively disproved an earlier assumption that a weedy race of *Helianthus bolanderi* had originated following the introgression of *H. annuus* genes into a serpentine race of *H. bolanderi* (Heiser, 1949), but instead was most probably of ancient origin (Rieseberg, Soltis and Palmer, 1988).

Discrepancies between phylogenies based on nuclear rDNA and cpDNA may also indicate past hybridization events. Phylogenetic analysis of restriction site variation of nuclear rDNA genes in the 21 taxa comprising *Helianthus* sect. *Helianthus* revealed some major discrepancies when compared with a phylogeny constructed from cpDNA analysis (Rieseberg, 1991). The rDNA evidence strongly suggested both recent and ancient introgression and provided compelling evidence that *H. anomalous*, *H. deserticola* and *H. paradoxus* were diploid species which originated as hybrids between *H. annuus* and *H. petiolaris*. Taken together, the cpDNA and rDNA data suggested that evolution in *Helianthus* has been reticulate rather than exclusively dichotomous and branching.

PCR methods in hybrid analysis

Restriction digestion of specific PCR amplified products (RFLP-PCR) of non-coding regions in the chloroplast genome (Taberlet *et al.*, 1991, Demesure, Sodzi and Petit; 1995) has recently been used in several studies of interspecific hybridization in plants. For example, Liston and Kadereit (1995) used RFLP-PCR to show that in the diploid species *Senecio flavus*, ssp. *breviflorus* and ssp. *flavus* have markedly different cpDNAs and that the cpDNA of ssp. *breviflorus* was most probably acquired through hybridization with a related diploid species. Using similar techniques, Ferris, King and Gray (1996) demonstrated that *Spartina alternifolia* was probably the maternal parent of *Spartina anglica*. Other PCR primers designed to amplify specific non-coding regions of the mitochondrial genome (Demesure, Sodzi and Petit, 1995), and the internal transcribed spacers (ITS) of nuclear ribosomal DNA (White *et al.*, 1990; used by Baldwin, 1992) are likely to be used in future analyses of plant hybridization.

The analysis of random amplified polymorphic DNA (RAPD, Williams *et al.*, 1990), generated by the polymerase chain reaction (PCR), offers great potential for distinguishing hybrids (Hadrys, Balick and Schierwater, 1992; Newbury and Ford-Lloyd, 1993; Weising *et al.*, 1995), and examining hybridization events. For example, RAPD analysis has been used to demonstrate that the putative allopolyploid, *Saxifraga osloensis*, is of hybrid origin between *S. tridactylites* and *S. adscendens* (Brochmann, Nilsson and Gabrielsen, 1996). The technique has also effectively demonstrated bi-directional introgression between *Iris fulva* and *I. hexagona* in mixed populations of *Iris fulva*, *I. hexagona* and *I. nelsonii* (Arnold, Buckner and Robinson, 1991), and that *I. nelsonii* is a product of hybridization involving *I. fulva*, *I. hexagona* and *I. brevicaulis* (Arnold, 1993). RAPD analysis has further confirmed an inter-generic hybrid speciation event between *Margyricarpus digynus* and *Acaena argentea*, which had been previously suspected from morphological evidence. The hybrid *Margyracaena skottsbergii* is endemic to the Juan Fernandez Islands and was found to exhibit an additive RAPD profile which combined all 18 fragments specific to *Acaena argentea* and all 23 unique fragments observed in *Margyricarpus digynus* (Crawford *et al.*, 1993).

Previous studies on York radiate groundsel

Previous studies by Warren (1987), Harris and Ingram (1992a) and Irwin and Abbott (1992) have investigated morphometric, isozyme and molecular variation in York radiate groundsel. A principal component analysis of 38 vegetative and floral characters conducted by Irwin and Abbott (1992), placed York radiate groundsel individuals, intermediate between *S. vulgaris* var. *vulgaris* and *S. squalidus* individuals

from York and Edinburgh populations (Irwin, 1990; Irwin and Abbott, 1992). This analysis also showed that York radiate groundsel was clearly distinct from individuals of *S. vulgaris* var. *hibernicus* from Edinburgh and that it possessed several novel characters, i.e. long calyculus bracts, a long, many lobed midleaf and two other midleaf characters, that were absent from the other taxa examined. Warren (1987) reported that York populations of radiate groundsel also had more pollen grains per floret and longer seeds than individuals of *S. vulgaris* var. *hibernicus* or var. *vulgaris* from other British populations, and possessed leaves that were highly dissected. A principal component analysis of nine morphometric characters conducted by Warren (1987) also placed York radiate groundsel intermediate to *S. vulgaris* var. *vulgaris* and *S. squalidus*.

Irwin and Abbott (1992) also examined isozyme variation in York radiate groundsel and its putative parental taxa. They found that of five isozyme loci that differentiated *S. vulgaris* from *S. squalidus*, York radiate groundsel possessed an *S. squalidus* phenotype at one locus (βEst-1a) and an *S. vulgaris* var. *vulgaris* phenotype at the other four loci (Acp-1a, αEst-1a, βEst-3a and Aat-3ab). A preliminary examination of cpDNA and rDNA RFLP variation further showed that York radiate groundsel possessed the rDNA profile typically found in British populations of *S. vulgaris* var. *vulgaris* and a cpDNA type shared by *S. vulgaris* and *S. squalidus* (Harris and Ingram, 1992a). Finally, Irwin and Abbott (1992) confirmed that all plants of York radiate groundsel were stable tetraploids (2n=40) forming regular bivalents during meiosis.

Objectives

The major objective of the work reported in this chapter was to use a broad range of analytical techniques to demonstrate that York radiate groundsel is of hybrid origin between *S. vulgaris* var. *vulgaris* and *S. squalidus*, and is distinct from the stabilized introgressant *S. vulgaris* var. *hibernicus*. To this end, a detailed examination of the respective morphologies of the taxa was conducted by means of single character and multivariate analysis. In addition, a chromosome analysis was completed and surveys of isozyme, rDNA, cpDNA and RAPD variation were carried out. In this way, the previous studies conducted by Irwin and Abbott (1992) and Harris and Ingram (1992a), that were aimed at characterizing York radiate groundsel with respect to its putative parents and *S. vulgaris* var. *hibernicus*, have been extended with larger sample sizes and new procedures of analysis.

Methods

Seed collection and plant propagation

Seed of *S. squalidus*, *S. vulgaris* (var. *vulgaris* and var. *hibernicus*) *S. cambrensis* and York radiate groundsel was collected from plants growing at locations listed in Table 2.1. Seed was sown onto damp filter paper and following germination, seedlings with a root of length approximately 1 cm were transplanted to 11.5 cm pots containing a 1:1 mix of Levingtons M2 compost to gravel. Plants were raised at ambient temperature in a glasshouse under 400 W mercury vapour lamps with the photoperiod set at 16 h.

Morphometric analysis

Two morphometric studies were undertaken; the first combined those characters used in previous studies (Crisp, 1972; Taylor, 1984; Warren, 1987; Irwin and Abbott, 1992) that were most informative in separating York radiate groundsel from parental and other hybrid *Senecio* taxa; the second study examined changes in leaf landmark positions, combined with the most informative floral characters from the first study. In the first study (referred to as m1 in Table 2.1), ten plants each of York *S. squalidus*, York *S. vulgaris* var. *vulgaris* and the York radiate groundsel (from Dalton Terrace, sampled in 1991), and six plants of *S. vulgaris* var. *vulgaris*, nine plants of *S. squalidus* and 12 plants of *S. vulgaris* var. *hibernicus* of Edinburgh origin were analysed. Plants were raised from seed in a fully randomized design. Plants were taken for first measurement on the day of full anthesis of the apical capitulum.

Twenty six characters (C1-C26), described in detail below, were measured on each plant, and represented a sample of the character sets examined by Crisp (1972), Taylor (1984), Warren (1987) and Irwin (1990). Fifteen of the characters recorded were descriptors of the capitulum, while 9 described vegetative traits, one plant fertility and one time to flowering. Characters C21, C22 and C25 were measured on each individual plant after an unopened capitulum had been covered with a small bag made of lens tissue and the plant returned to the glasshouse until achenes had ripened. The midleaf area and perimeter used to calculate characters C18-C20 were measured using a Delta-T area meter (Delta-T Devices, Cambridge).

In the second morphological study (referred to as m2 in Table 2.1), 19 plants of *S. vulgaris* var. *vulgaris* of Methil origin and 14 from York, together with five plants of *S. vulgaris* var. *hibernicus* from Edinburgh, 40 plants of the York radiate groundsel and 19 plants of *S. squalidus* also of York origin were examined. Plants were raised from seed collected from two populations of the York radiate groundsel (20 plants

Table 2.1. Locations sampled and types of analysis conducted on material of *S. vulgaris, S. squalidus*, and the York radiate groundsel.

Taxa	Location		Types of analysis [a]
S. vulgaris var *vulgaris*	England	Bristol	i(4), r, c
		York	m1(10), m2(14), i(109), r(9), c(9), p(5)
	Scotland	Aberfeldy	i(4), r, c
		Bo'ness	i(3), r, c
		Dundee	i(4), r, c
		Leith, Edinburgh	m1(6), i(20), r, c
		Fort William	r, c
		Glasgow	r, c
		Kingussie	r, c
		Kirriemuir	r, c
		Lairg	i(2), r(2), c(2)
		Letham Angus	i(4), r, c
		Markinch	i(4), r, c
		Methil	m2(19), i(4), r, c, p(8)
		Perth	r, c
		Stranraer	r, c
		Ullapool	r, c
	Wales	Barry	r, c
		Brymbo, Wrexham	r, c
		Ffrith, Wrexham	r, c
		Southsea, Wrexham	i(4), r, c
		Wrexham	r, c
	Eire	Cork	r, c
		Passage West, Cork	i(9), c
	France	Brittany	r, c
S. vulgaris var. *hibernicus*	England	Bristol	i(3), r, c
	Scotland	Leith, Edinburgh	m1(12), m2(5), i(19), r, c, p(5)
		Glasgow	i, r(2), c(2)
		Grangemouth	i(8)
	Wales	Bangor	i(2)
		Barry	i(6)
		Cardiff	r, c
		Mochdre	i(10), r, c
		Broughton, Wrexham	i(6), r, c
		Brymbo, Wrexham	i, r, c
		Ffrith, Wrexham	i(3), r, c
		Rhostyllen, Wrexham	i(11), r, c
		Southsea, Wrexham	i, r, c
		Wrexham	i(2), r, c
	Eire	Cork	i(14), r, c
		Passage West, Cork	i(17), r(3), c(4)
York radiate groundsel	England	Dalton Terrace, York	m1(10), m2(20), i(117), r(2), c(2)
		Lendal Bridge, York	m2(20), ch(11), i(79), r(5), c(5), p(11)

Table 2.1. Continued.

Taxa	Location	Types of analysis [a]
S. cambrensis	Scotland Leith, Edinburgh	i (17)
	Wales Mochdre	i (28)
	Wrexham	i (21)
S. squalidus	England York	m1(10), m2(19), i(14), r(2), c(2), p(3)
	Scotland Leith, Edinburgh	m1(9), i(12), p
	Eire Cork	i, p

[a] m1 = first morphometric analysis; m2 = second morphometric analysis; ch = chromosome analysis; i = isozyme analysis; c = cpDNA analysis; r = rDNA analysis; p = RAPD-PCR analysis. Values placed in parentheses indicate the number of individuals greater than one, examined in a particular analysis

from the Dalton Terrace site sampled in 1993 and 20 from Lendal bridge, sampled in 1991), two populations of *S. vulgaris* var. *vulgaris* (one from Methil in Scotland, the other from York) and one population of each of *S. vulgaris* var. *hibernicus* and *S. squalidus*. Plants were again grown in a fully randomized design and taken for first measurement on the day of anthesis of the central disc floret of the apical capitulum.

Twenty characters were measured initially on each plant, representing a subset of the first morphometric character set, but included three additional characters (C27-C29). Fourteen of the characters recorded were descriptors of the capitulum (C2-C4, C6, C8-C11, C21-C23 and, C27-C29), while five described vegetative traits (C1, C15, C18, C19 and C20), and one the time to apical anthesis (C26). Character C28 was measured on a mature, but unopened, disc floret near the centre of the apical capitulum and character C29 was measured, where possible, on the most centrally located disc floret of the apical capitulum. A further 11 linear and seven angular midleaf characters were also measured in the second study. However, instead of direct measurement using callipers or a protractor, the character magnitude was calculated from the x and y coordinates of key landmark positions on the midleaf. A total of 23 midleaf landmarks were defined according to the principles used by Hill (1980) and Jensen (1990), to ensure that the position of identical anatomical structures could be identified confidently between the different *Senecio* taxa used in the study. Each landmark position was recorded by placing a 1 mm square grid, printed onto a transparent sheet, over the top of the flattened midleaf. A zero x and y coordinate point was assigned on the grid and aligned with the point of midleaf stem attachment, and the primary vein was straightened from base to apex to align with the y zero axis. Each landmark was recorded as an x and y coordinate to the nearest 0.5 mm, where positive x coordinates indicated landmarks to the right of the primary vein and negative ones to the left (Table, 2.2). The landmarks were entered into a MINITAB spread sheet and linear distances (C30-C41) and angular measurements (C42-C48) were calculated according to the equations shown below for each of these characters.

The Character set

C1 Plant height.

Length from the base of the stem, defined as the cotyledon node, to the level of the stigma of the apical capitulum at anthesis.

C2 Inflorescence length.

Length of the apical stem node, defined as the node subtending the apical capitulum, to the level of the stigma of the apical capitulum at anthesis (see Figure 2.1a).

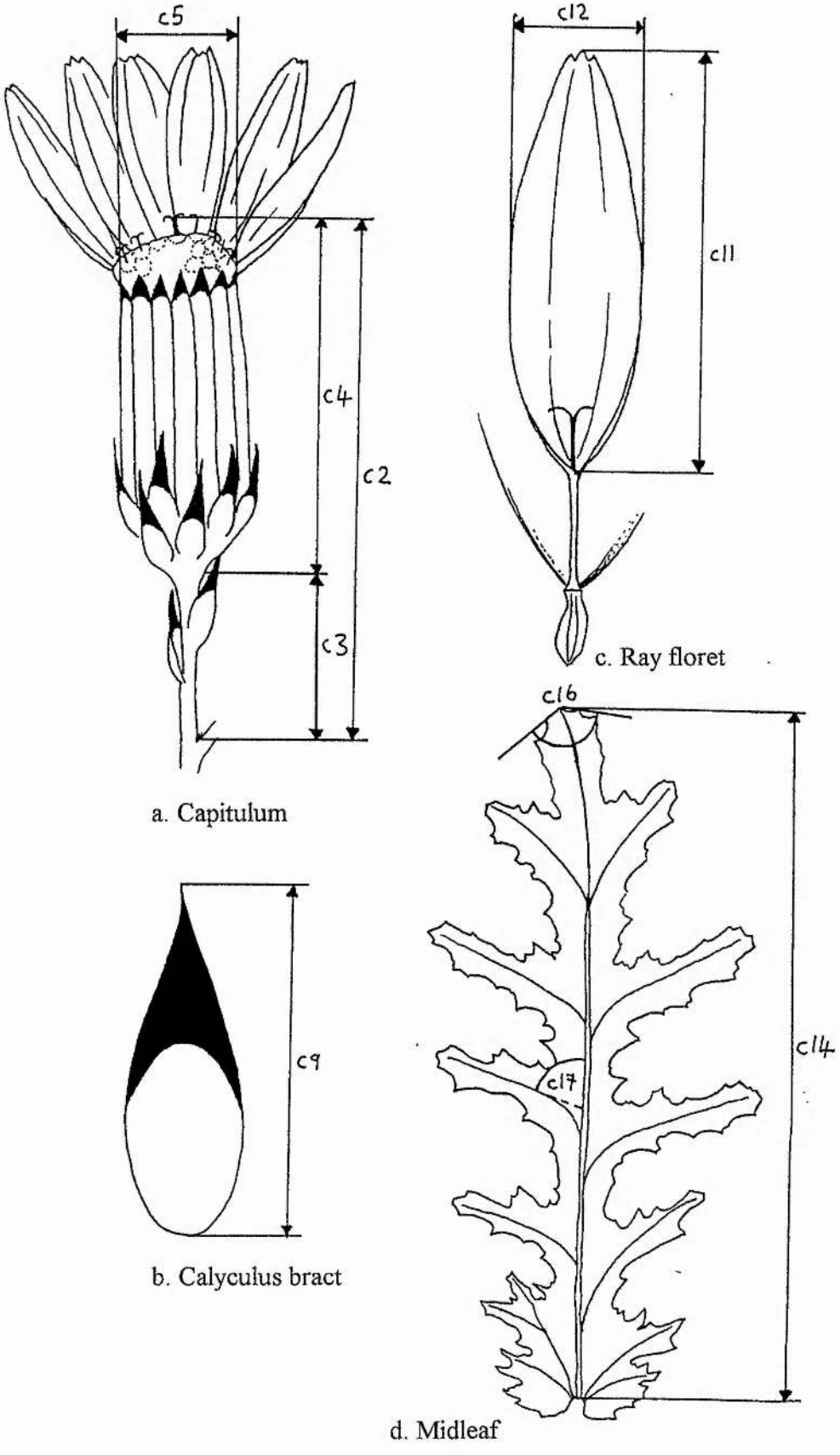


Figure 2.1. The apical capitulum and midleaf of *S. vulgaris* (after Taylor, 1984) showing; a. Capitulum size characters C2-C5; b. Calyculus bract character C9; c. Ray floret characters C11 and C12; d. Midleaf characters C14, C16 and C17.

C3 Peduncle length.

Length of the peduncle from the apical stem node to the point at which the peduncle widens into the receptacle (see Figure 2.1a).

C4 Capitulum length.

Length from the point at which the peduncle widens into the receptacle to the end of the stigma of the central ray floret of the apical capitulum (see Figure 2.1a).

C5 Capitulum width.

Diameter of the apical capitulum, measured at the end of the capitulum prior to capitulum widening caused by ray floret subtension (see Figure 2.1a).

C6 Number of phyllaries.

C7 Proportion of phyllaries with black tips.

Defined as the number of phyllaries with black or brown tips divided by the total number of phyllaries.

C8 Number of calyculus bracts.

Total number of bracts which are attached to the receptacle above the point at which the peduncle widens.

C9 Mean calyculus bract length.

Defined as the sum of the lengths of the calyculus bracts, see Figure 2.1b, divided by the total number of calyculus bracts.

C10 Number of ray florets.

C11 Mean outer floret length.

Defined as the sum of the lengths of the outer (ray) florets, measured from the base to the apex of the ligule, divided by the total number of outer (ray) florets (see Figure 2.1c).

C12 Mean outer floret width.

Defined as the sum of the maximum widths of the outer (ray) florets (see Figure 2.1c), divided by the total number of outer (ray) florets.

C13 Longest leaf length.

Length of the longest leaf measured parallel to the primary vein.

C14 Midleaf length.

Maximum length of the midleaf, defined as the leaf attached to the stem nearest to the midpoint of the plant height (C1). Measured parallel to the primary vein (see Figure 2.1d).

C15 Number of midleaf lobes.

The number of secondary veins which supply defined lobes plus the apical lobe. The apical lobe is defined as originating at the point at which the secondary veins are of equal thickness to the primary veins. Lobes in the basal lamina were not counted if the secondary vein originated from the primary vein before the point of leaf attachment to the stem. The definition of the auricle being the part of the basal lamina in which the veins originate in the stem.

C16 Midleaf apical angle.

Defined as the angle between the apex of the primary vein and the apices of the adjacent marginal tooth sinuses (see Figure 2.1d).

C17 Mid-lobe secondary vein angle.

Defined as the angle between the secondary vein of the lobe closest to the midpoint of the midleaf and the primary vein (see Figure 2.1d).

C18 Leaf dissection.

Defined as the ratio of the perimeter of the midleaf divided by the square root of the area.

C19 Standardized leaf perimeter.

Defined as the perimeter of the midleaf divided by the midleaf length (C14).

C20 Standardized square of leaf area.

Defined as the square root of the area of the midleaf divided by the midleaf length (C14).

C21 Mean seed length.

Defined as the sum of the lengths of five achenes collected from the second most apical capitulum divided by five.

C22 Total number of seeds per capitulum.

The total number of achenes (fertile and sterile) found in the ripe, second most apical capitulum.

C23 Number of pollen pores.

The average number of pollen pores observed under light microscopy on at least ten fully stained pollen grains from the apical capitulum.

C24 Pollen pore size.

The mean of two pollen pore diameters from two separate fully stained pollen grains from the apical capitulum.

C25 Proportion of self-seed set per capitulum.

Defined as the number of fertile achenes in the second most apical capitulum bagged before anthesis divided by the total number of achenes in the ripened capitulum (C22).

C26 Time to apical capitulum anthesis.

Defined as the number of days from seed germination to the anthesis of the centre disc floret of the apical capitulum.

C27 Number of peduncle bracts.

Total number of bracts which are attached to the peduncle, below the point at which the peduncle widens into the receptacle.

C28 Total number of pollen grains.

The number of pollen grains held in all stamens in a fully developed but unopened centrally placed disc floret of the apical capitulum.

C29 Number of stigmatic papillae.

The number of stigmatic papillae observed under a light microscope on both fronds of a stigma from a centrally located ray floret in the apical capitulum.

Character set calculated from landmark coordinates

Refer to Table 2.2 and Figure 2.2, for definition of leaf landmarks, where midleaf linear and angular measurements are calculated from the x and y coordinates of relative landmarks. See Figure 2.3a for diagrammatic representation of linear measurements and Figure 2.3b for angular measurements.

C30 Midleaf length. Identical to C14. Calculated as 1y from Figure 2.2, shown in Figure 2.3a.

Table 2.2 Definition of leaf landmarks shown in Figure 2.2.

1 Apex of primary vein.

2 Apex of right marginal tooth sinus adjacent to primary vein apex.

3 Apex of left marginal tooth sinus adjacent to primary vein apex.

4 Point of exmedial curvature of right apical lobe leaf margin.

5 Point of exmedial curvature of left apical lobe leaf margin.

6 Apex of right secondary vein adjacent to primary vein.

7 Apex of left secondary vein adjacent to primary vein.

8 Intersection of most apical secondary vein with primary vein.

9 Mid lobe apex

10 Intersection of mid-lobe secondary vein with primary vein, ignoring first 10% of vein due to excessive curvature.

11 Mid-lobe exmedial curvature of top leaf margin.

12 Lower mid-lobe leaf margin edge directly below L11 measured parallel to primary vein.

13 Apex of top marginal tooth sinus adjacent to mid-lobe apex.

14 Apex of bottom marginal tooth sinus adjacent to mid-lobe apex.

15 Base of primary vein, point of stem attachment. This is the zero point for x and y axes.

16 Base of auricle right of the primary vein.

17 Base of auricle left of the primary vein.

18 Point of maximum extension of auricle right from the primary vein.

19 Point of maximum extension of auricle left from the primary vein.

20 Maximum exmedial leaf margin curvature above basal auricle and below first right hand secondary vein.

21 Maximum exmedial leaf margin curvature above basal auricle and below first left hand secondary vein.

22 Apex of right hand secondary vein, adjacent to the basal auricle.

23 Apex of left hand secondary vein, adjacent to the basal auricle.

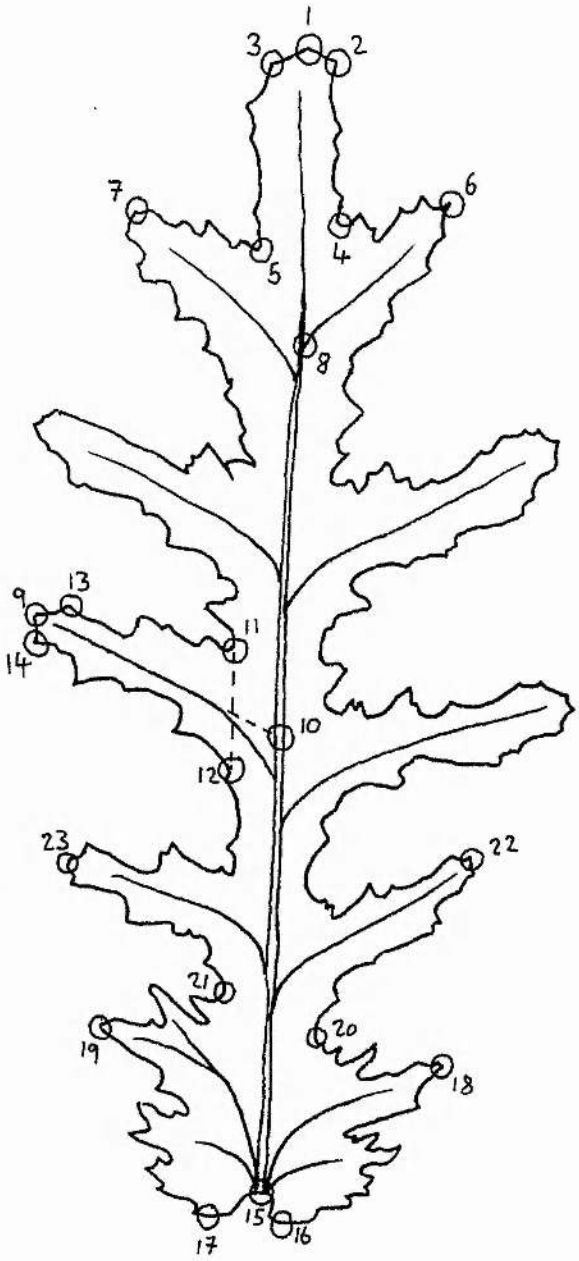
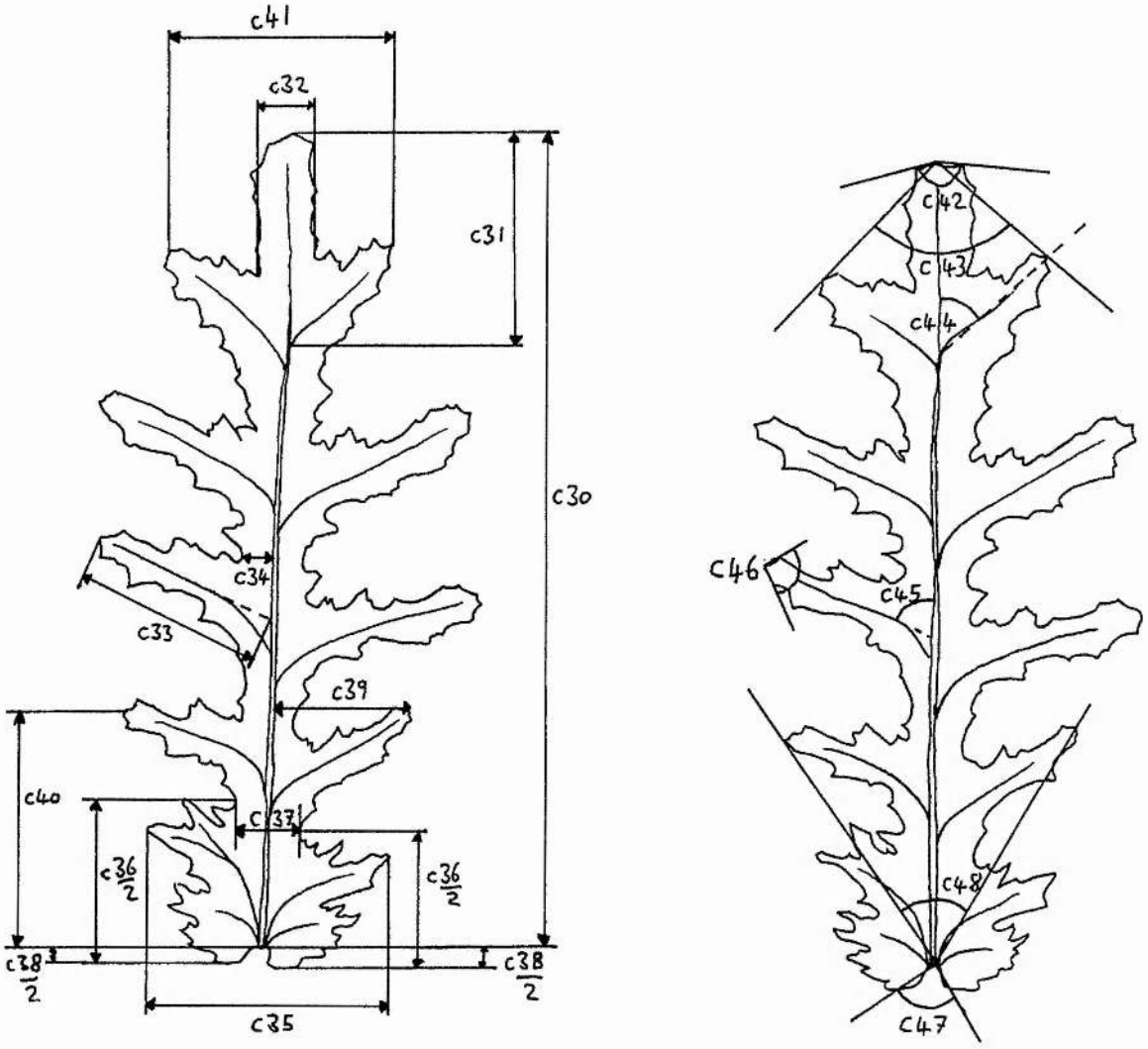


Figure 2.2 The midleaf of *S. vulgaris* showing all 23 midleaf landmark positions.



a. Linear measurements

b. Angular measurements

Figure 2.3 The midleaf of *S. vulgaris* showing; a. linear measurements of midleaf derived from landmark coordinates; b. angular measurements of midleaf derived from landmark coordinates.

C31 Apical lobe length.

Length of the apical lobe measured along the primary vein from the point of origin of the most apical secondary vein to the apex of the lobe. Calculated as 1y-8y from Figure 2.2, shown in Figure 2.3a.

C32 Apical lobe basal width.

The width of the apical lobe at the point of maximum exmedial curvature of the base of the primary lobe defining the first left and right non-apical lobes, measured perpendicular to the primary vein. Calculated as 4x+5x from Figure 2.2, shown in Figure 2.3a.

C33 Mid-lobe vein length.

The length of the secondary vein occurring nearest to the midpoint of the primary vein, ignoring the first 10% of secondary vein due to excessive curvature. Calculated as $((9x)^2+(9y-10y)^2)^{1/2}$ from Figure 2.2, shown in Figure 2.3a.

C34 Mid-lobe lamina width.

The width of the primary lamina, from the centre of the primary vein to the point of maximum exmedial curvature of the top leaf margin edge of the mid-lobe. Measured perpendicular to the primary vein. Calculated as 11x from Figure 2.2, shown in Figure 2.3a.

C35 Basal auricle width.

Maximum width of the midleaf auricle, measured perpendicular to the primary vein. Calculated as 19x+18x from Figure 2.2, shown in Figure 2.3a.

C36 Mean basal auricle height.

The height of the basal auricle averaged for measurements from both sides of the primary vein. Calculated as ((17y+16y)/2)+((20y+21y)/2) from Figure 2.2, shown in Figure 2.3a.

C37 Basal lamina width.

The minimum width of the lamina, from leaf margin to leaf margin perpendicular to the primary vein, immediately above the basal auricle and before the first basal lobes. Calculated as 21x+20x from Figure 2.2, shown in Figure 2.3a.

C38 Basal auricle extension beyond stem attachment.

The average length of the free portion of the basal auricle beyond the point of stem attachment. Calculated as (16y+17y)/2 from Figure 2.2, shown in Figure 2.3a.

C39 Right basal lobe width.

The width, perpendicular to the primary vein, of the first true lobe after the basal auricle. Calculated as 22x from Figure 2.2, shown in Figure 2.3a.

C40 Height of left basal lobe.

The height from the point of stem attachment to the apex of the first true lobe after the basal auricle, measured parallel to the primary vein. Calculated as 23y from Figure 2.2, shown in Figure 2.3a.

C41 Width of two apical lobes.

The distance, perpendicular to the primary vein, between the apices of the two most apical lobes, excluding the apical lobe. Calculated as 6x+7x from Figure 2.2, shown in Figure 2.3a.

C42 Apical angle A. Identical to C16.

Calculated as (arctan(3x/(1y-3y)))+(arctan(2x/(1y-2y))) from Figure 2.2, shown in Figure 2.3b.

C43 Apical angle B.

The angle between the apex of the primary vein and the apices of the adjacent secondary veins. Calculated as (arctan(7x/(1y-7y)))+(arctan(6x/(1y-6y))) from Figure 2.2, shown in Figure 2.3b.

C44 Secondary vein angle of apical adjacent lobe.

The angle between the primary vein and the adjacent most apical secondary vein. Calculated as arctan (6x/(6y-8y)) from Figure 2.2, shown in Figure 2.3b.

C45 Mid-lobe secondary vein angle. Identical to C17.

Calculated as arctan (9x/(9y-10y)) from Figure 2.2, shown in Figure 2.3b.

C46 Mid-lobe apical angle.

The angle between the apex of the mid-lobe secondary vein and the apices of adjacent marginal tooth sinuses. Calculated as (arctan((13y-9y)/(9x-13x)))+(arctan((9y-14y)/(9x-14x))) from Figure 2.2, shown in Figure 2.3b.

C47 Basal angle A.

The angle between the base of the primary vein at the point of stem attachment and the most basal auricle lobes on either side of the primary vein. Calculated as (arctan(16x/16y))+(arctan(17x/17y)) from Figure 2.2, shown in Figure 2.3b.

C48 Basal angle B.

The angle between the base of the primary vein and the apices of the adjacent basal lobes on either side of the primary vein. Calculated as (arctan(22x/22y))+ (arctan(23x/23y)) from Figure 2.2, shown in Figure 2.3b.

Multivariate statistical procedures

Principal component analysis is a multivariate technique for examining relationships among several quantitative variables and finds linear combinations of a set of variates that maximize the variation contained within them, thereby displaying most of the original variability in a smaller number of dimensions (SAS, 1990). Plots of principal components are especially valuable tools in exploratory data analysis and were used for initial examination of morphometric data. Principal components are linear combinations of the original variables, with coefficients equal to the eigenvectors of the correlation or covariance matrix, and are sorted by descending order of eigen values, which are equal to the variances of the components. In addition, eigen values are orthogonal and principal component scores are jointly uncorrelated, although these two properties are quite distinct (Manly, 1992).

Canonical variate analysis (CVA) was used to distinguish between taxa that fell into natural groupings, and the analysis operates on a within-group sums of squares and products matrix. The within-group sums of squares and products are pooled over all groups and from the group means and sizes the between-group sums of squares and products are calculated (SAS, 1990). The derivative finds linear combinations of the original variables that maximize the ratio of between-group to within-group variation, thereby giving functions of the original variables that can be used to discriminate between the groups. The squared distances between group means are Mahalanobis D^2 statistics when all dimensions are used; otherwise they are approximations. Discriminant function analysis was further used to assign units to groups that have previously been discriminated by CVA following comparison of all unit canonical axes to group variances (see Chapter 3; Manly, 1992).

Principal coordinate analysis, or metric scaling, is an ordination method which operates with data in the form of a symmetric matrix of associations. This is unlike principal component analysis which operates with data in the form of a data matrix or a two-way table, and is particularly useful when data are non-normal or heteroscedastic, e.g. RAPD fragment presence/absences data (see later), when a distance symmetric matrix can be calculated from the raw data (Adams, 1995). Given

a symmetric matrix, e.g. genetic distances, with values representing the associations amongst a set of *n* units, principal coordinates analysis following Gower (1966), attempts to find a set of points for the *n* units in a multidimensional space and places similar units close together and dissimilar units further apart. The coordinates of the points are arranged so that their centroid is at the origin. Furthermore they are arranged relative to their principal axes, so that the first dimension of the solution gives the best one-dimensional fit to the full set of points, and so on for subsequent dimensions. Associated with each dimension of the set of coordinates is a latent root which is the sum of squares of the coordinates of all the points in that dimension. For *n* units, if there is an exact solution it will be in at most *n*-1 dimensions. However, such a solution is not always available, because the matrix of distances derived from the associations may not be Euclidean: that is, the distances may not be reproducible by points in a Euclidean space of any number of dimensions. If an incomplete solution results, either because the Euclidean property does not hold or because not all dimensions are used, then a residual can be calculated for each point. This residual is the difference between the distance from the point for that unit in the incomplete solution to the centroid and the equivalent distance derived from the original data, some of the residuals may be represented by complex numbers (SAS, 1990).

Statistical analysis of morphometric character values

The data sets obtained from both studies were independently analysed by means of a one-way ANOVA to examine differences between means for each character in turn using Tukey-Kramer multiple comparison. Before ANOVA, data were tested for normality and heteroscedasticity and those not conforming were transformed (Sokal and Rohlf, 1981). For the second data set the means of individuals from each of the two populations of York radiate groundsel (Lendal Bridge and Dalton Terrace) were compared for each character in turn by t-test. No significant difference was found between the two populations for any character and data from these populations were pooled before ANOVA.

In preparation for canonical variate analysis, characters for which there was no significant difference between taxa (C4 and C26) and those characters that had zero standard deviation (C10 and C23) were removed. Canonical variate analysis (CVA) (Manly, 1992) followed the CANDISC procedure of SAS (SAS Institute, Inc., 1990). Characters were used to characterize each taxon (specified as groups) after verifying that the variables were multivariate normally distributed and assuming equal variances and covariances in each group.

For the first morphometric data set, Mahalanobis' distances were calculated using group means and variances, and a dendrogram was constructed from the distance matrix by the unweighted pair-group method with arithmetic means (UPGMA; Sneath and Sokal, 1973). The 39 characters in the second morphometric data set were split into two groups. One group of 16 measurements mainly described floral morphology (C1-C4, C6, C8-C11, C21-C23 and C26-C29), while the other group of 23 measurements described leaf morphology (C15, C18-C20 and C30-C48). Both floral and leaf character sub-sets were then subjected to canonical variate analysis to examine the relative influence of these two suits of characters on the morphometric separation of taxa.

Examination of midleaf landmarks

To examine midleaf shape in more detail, the landmark data were subjected to a generalized resistance fit analysis using the program GRF (Rohlf and Slice, 1991). This is an iterative procedure that fits an entire sample of landmarks to an estimated average position for each landmark. This mean landmark position for each point is referred to as the consensus landmark position and a standard deviation estimate for each point is also made. The approach uses median-based estimates and prevents the results being unduly affected by a small subset of landmarks (Slice 1992).

The consensus landmark positions for the hybrid taxa (*S. vulgaris* var. *hibernicus* and York radiate groundsel) were compared to the consensus landmark reference positions of the two parental taxa (*S. vulgaris* var. *vulgaris* and *S. squalidus*) using an ordinary resistant fitting procedure. This method fits the consensus landmark positions of the hybrid to the reference consensus positions of each of the parental taxa. The method uses a median-based method to estimate the superimposition of parameters and expresses the fit of the subject to the reference as the mean squares of the difference between all consensus landmarks.

Chromosome analysis

Eleven plants of the York radiate groundsel raised from seed collected at Lendal Bridge, York in 1991, were examined for mitotic chromosome behaviour and karyotype markers. Several chromosome counts were made for each individual to confirm their chromosome number.

Mitotic chromosome counts

The method used is outlined in Jong (1994). Active white root tips were harvested at 12 noon and placed in fresh pre-treatment solution of 0.002M 8-hydroxyquinoline for

twenty hours at 10-15o C. Bromo-naphthalene and colchicine were also used as pre-treatments, but either gave poorly condensed chromosomes or caused clumping of condensed chromosomes. Root tips were washed and fixed overnight in freshly prepared Farmer's fluid (3 parts ethanol to one part glacial acetic acid). Root tips were also stored in this fixative. For staining, tips were washed in two changes of distilled water and placed in prepared Feulgens reagent for 30 minutes. Feulgens reagent was prepared by mixing 0.7 g basic fuchsin/p-rosaniline and 3.8 g sodium metabisulphate in 200 ml of 0.15 N HCl for 2-3 hours at room temperature. This solution was de-colourized by shaking with 1 g activated charcoal, before filtering and making up to 200 ml with distilled water. The solution prepared in this way, which was approximately of pH 2.2, was stored refrigerated in the dark in an airtight bottle. After staining, root tips were washed in distilled water and hydrolysed in 5 N HCl for 45 minutes. Root tips were washed again and digested in 4% pectinase for 10 minutes. The densely stained tissue (i.e. apical region of root tip) was placed on a clean slide in a drop of 45% acetic acid; tissue was tapped with a blunt metal rod and covered with a clean No. 1 coverslip. The coverslip was covered with blotting paper and firmly pressed down onto the slide. The slide was gently heated before sealing the coverslip with nail varnish. Cells were viewed under oil emersion with a 100 x objective using a normal or phase contrast light microscope.

Isozyme analysis

A survey of isozyme variation was conducted on plants raised from two populations of York radiate groundsel, 13 populations of *S. vulgaris* var. *vulgaris* (including two populations from York), 15 populations of *S. vulgaris* var. *hibernicus*, three populations of *S. squalidus* and three populations of *S. cambrensis* (Table 2.1). Horizontal starch gel electrophoresis was conducted on crude protein extracts of leaf or flower bud tissue with the following enzyme systems assayed: aconitase (ACO), aspartate aminotransferase (AAT), α esterase (αEST), β esterase (βEST) Isocitrate dehydrogenase (IDH), Acid phosphotase (ACP) and glutamate dehydrogenase (GDH).

Starch gel electrophoresis

Horizontal starch gel electrophoresis was carried out using the electrophoretic and staining procedures of Ashton (1990). A small amount of apical bud tissue was removed and homogenized on ice in a few drops of extraction buffer (0.2 M Tris-HCl pH 8.0, 0.49 mM KCl, 0.049 mM $MgCl_2$, 0.047 mM EDTA Na_4, 50% (w/v) PVPP, 0.5 µl Triton x-100, 4% (v/v) β-mercaptoethanol), to form a crude protein extract. Starch gels (11% w/v) were prepared using the appropriate gel buffer (for composition see Appendix 1), by heating the solution until starch dissolved and thickened. The

molten solution was then degassed and poured into a gel mould (20 x 22 cm). Once cool, wells were made in gels for loading with filter paper squares soaked in crude protein extract. A drop of bromophenol blue was placed on each outer lane to act as a tracker dye. Gels were then placed on a gel rig containing the appropriate electrode buffer (see Appendix 1) and were run at a constant voltage of 250 V (70 mA) for 3-4 hours at 4o C. Once the gel front had migrated 8 cm from the origin, gels were trimmed and sliced horizontally into three layers. Each gel slice was transferred to a different enzyme staining solution (see below) and incubated at 37o C in the dark for up to 2 hours. After staining was complete, gel slices were briefly rinsed in water and fixed in 50% glycerol before photographing and scoring individuals for allozyme phenotype.

Analysis of allozyme phenotype frequencies

Material of *S. vulgaris* var. *vulgaris* (except York material) and var. *hibernicus* from populations in England and Wales, and Scotland were pooled and allozyme phenotypic frequencies recalculated. Nei's genetic distance (1972) was calculated between all population pairs from allozyme phenotype frequencies. From this matrix a UPGMA dendrogram was constructed using BIOSYS-1 (Swofford and Selander, 1981). It was assumed that tetraploids and hexaploids exhibited disomic inheritance of allozyme variants, and so fixed heterozygous genotypes were coded as single locus diallelic heterozygotes.

RFLP analysis of Chloroplast DNA (cpDNA) and nuclear ribosomal DNA (rDNA)

A restriction analysis of cpDNA and nuclear rDNA variation was conducted on 33 individuals (34 for cpDNA) of *S. vulgaris* var. *vulgaris*, 16 individuals (17 for cpDNA) of *S. vulgaris* var. *hibernicus*, seven York radiate groundsel individuals and two *S. squalidus* individuals from York (Table 2.1). Material of York radiate groundsel was collected from two main sites in York, Dalton Terrace and Lendal Bridge, whilst material of *S. vulgaris* was cultivated from seed sampled from a wide range of populations located in the British Isles, but included nine samples of *S. vulgaris* var. *vulgaris* from around York. For comparative purposes the results of a previous restriction analysis of plants of *S. squalidus* representing a wide range of populations from the British Isles (Abbott, Curnow and Irwin, 1995; and Curnow unpublished) were made available for analysis.

Sampling of Leaf tissue for DNA extraction

Approximately 2g of leaf tissue was taken from each plant aged 10-12 weeks, which had been raised from seed under glass and de-starched overnight by storing in the dark at 4o C. Leaf material was either used directly or kept frozen at -20o C until used.

Large scale DNA extraction and purification

The procedure followed that of Doyle and Doyle (1987), and Sambrook, Fritsch and Maniatus (1989). Two grams of fresh or frozen leaf material was ground to a fine powder in liquid nitrogen. Twenty ml of 2 x CTAB extraction buffer (2% (w/v) CTAB, 100 mM Tris, 20 mM EDTA Na_2, 1.4 M NaCl, 1% (w/v) PVP 40-T, to pH 8.0 with HCl, plus 0.2% β-mercaptoethanol after autoclaving) preheated to 60o C, were added to the powder and the mix was incubated at 60o C with occasional swirling for 30 minutes. After cooling to room temperature, 10 ml of chloroform (24:1 chloroform:isoamyl alcohol) were added and mixed to a single phase then centrifuged at 8,000 rpm for 10 min at room temperature. The aqueous layer was removed to a new tube, mixed with 2/3 volume (13 ml) propan-2-ol at -20o C and left to stand at room temperature for at least one hour. The precipitate was recovered by centrifugation at 5,000 rpm for 10 minutes at room temperature. The supernatant was discarded and the pellet was briefly air dried before washing in 10 ml wash buffer (75% ethanol, 10 mM ammonium acetate) for at least one hour. The pellet was spun down at 8,000 rpm at room temperature for 10 minutes before discarding the supernatant and drying the pellet at room temperature for 15 minutes. Excess wash buffer was wiped from the sides of tube with a tissue and the pellet was dissolved in 8 ml TE (10 mM Tris, 1 mM EDTA Na_2, to pH 7.6 with HCl). For purification, this solution was combined with 8.625 g caesium chloride and 115 µl 10 µg/ml ethidium bromide and made up to 11.5 ml in a Sorvall ultracrimp ultracentrifuge tube. The DNA sample was ultracentrifuged for 18-24 hours at 20o C at 53,000 rpm in a Sorvall T-865.1 rotor. After centrifugation, DNA which was clearly visible as a fluorescent band under UV illumination, was recovered using a hypodermic syringe and transferred to a 15 ml glass centrifuge tube. To remove ethidium bromide from the sample, 3 ml TE-saturated butan-1-ol was added to the tube and shaken vigorously. The upper layer was removed with a Pasteur pipette and discarded before repeating the step again. The DNA solution was then combined with an equal or greater volume of TE and 2 volumes of propan-2-ol in a 30 ml centrifuge tube. The sample was centrifuged at 9,500 rpm at room temperature for 30 minutes and the supernatant discarded. The DNA pellet was air dried and then dissolved in 1 ml TE before transferring to two, 1.5 ml microfuge tubes. To each tube, 170 µl of 7.5 M ammonium acetate were added followed by 1 ml propan-2-ol (at -20o C), before

mixing by inversion and storing overnight at -20° C. DNA was pelleted by spinning at 13,000 rpm at room temperature for 30 minutes. The supernatant was discarded and the pellet air dried. DNA pellets were re-dissolved in TE and combined together in a total volume of 0.5 ml, followed by the addition of 170 µl ammonium acetate and 1 ml propan-2-ol (at -20° C) and inversion and storage at -20° C overnight. DNA was pelleted by spinning at 13,000 rpm at room temperature for 30 minutes and the pellet was allowed to air dry. The DNA pellet was finally redissolved in a volume of 100 µl TE. The concentration and quality of DNA in this solution was determined by agarose gel electrophoresis (see below) before diluting the concentration to 25 ng/µl and storing at -20° C before use.

Determination of DNA concentration by agarose gel electrophoresis

To check the concentration of DNA, samples were separated by electrophoresis on a 0.8% (w/v) agarose gel. For large gels, 2.4 g agarose was added to 300 ml of 1 x TAE (0.04 M Tris, 0.02 M sodium acetate, 1 mM EDTA), and for small gels, 0.8 g agarose was added to 100 ml of 1 x TAE. The solution was brought to the boil and simmered for a few minutes until 'lens like' particles had dissolved. The solution was left to cool to around 60° C before 0.5 µg/ml ethidium bromide was added and the solution poured into a gel mould and well combs put in place. Once set, the gel was placed in a gel rig and covered with 1 x TAE. Two µl of DNA solution were combined with 8 µl distilled water and 1 µl of 10 x gel loading buffer (0.125 M EDTA, 50% glycerol, 0.1% SDS and 2 mg/ml bromophenol blue). DNA samples were loaded into the wells of the gel along with a concentration marker (250 ng calf thymus DNA) in the outer lanes. Gels were run at 100 mA for 1 to 2 hours before visualising under UV illumination and estimating the quantity of DNA present in each sample relative to the 250 ng standard.

Restriction digestion of DNA

Digests were usually carried out with 125-250 ng DNA, 10 units of restriction enzyme per µg DNA and sterile distilled water up to a total reaction volume of 15 µl. The restriction enzymes targeted their respective cutting sites and recommended temperatures for digestion were as follows; *Bcl*I (TGATCA) 50° C; *Bgl*II (AGATCT) 37° C; *Cla*I (ATCGAT) 37° C; *Eco*RI (GAATTC) 37° C; *Eco*RV (GATATC) 37° C; *Hae*III (GGCC) 37° C; *Hin*DIII (AAGCTT) 37° C; *Pst*I (CTGCAG) 37° C; *Pvu*II (CAGCTG) 37° C; *Sac*I (GAGCTC) 37° C. Digests were set up on ice in 0.5 ml microfuge tubes. Lids were sealed and tubes were incubated overnight at the recommended temperature. After digestion 1/10th volume of gel loading buffer was

added to stop the reaction and to prepare the samples for electrophoretic separation on an agarose gel.

Agarose Gel electrophoresis of digested fragments

Following restriction digestion, samples were loaded onto a 1% (w/v) agarose gel with a molecular weight marker (*Hin*DIII digested lambda phage DNA, fragment sizes in Kb; 23.130, 9.416, 6.557, 4.361, 2.322, 2.027, 0.564 and 0.125) loaded into the two outermost lanes for later fragment sizing. Gels were run at 90 mA for six hours. Gels were generally run to within 4-5 cm of the end of the gels and then examined under transmitted UV light and photographed (usually aperture f 5.6 and shutter speed 0.25-0.5 seconds) using an orange filter and Polaroid 667 film. A UV transparent ruler was placed alongside the size markers so the distance that fragments migrated could be measured.

Southern blotting of Gels onto membranes

Southern blotting of gels followed the procedures of Southern (1975) and Sambrook, Fritsch and Maniatus (1989) on nitro-cellulose membranes. The agarose gel containing digested fragments, was trimmed along the bottom of the wells and washed in denaturation buffer (1.5 M NaCl and O.5 M NaOH) for 30 minutes with gentle agitation, followed by a brief rinse in distilled water. The gel was then neutralized by immersing in neutralization buffer (1.5 M NaCl, 1 mM EDTA Na_2 and 0.5 M Tris-HCl) for 30 minutes with gentle agitation. The Southern blot was assembled using 20 x SSC (3 M NaCl and 0.3 M trisodium citrate) as the transfer buffer. The gel was turned upside down and the edge of the membrane carefully aligned with the gel well origin. The membrane was covered with 3MM blotting paper and fresh paper towels, and DNA transfer was left to proceed for 16-20 hours. The blot was dismantled and the membrane briefly rinsed in 2 x SSC to remove any adhering agarose before drying on paper towels. The DNA was bound to the nitro-cellulose filters by UV light cross linking for 2-4 minutes on a UV light transilluminater before storing at room temperature until used.

Preparation of probes

Filters were probed with cpDNA and rDNA cloned fragments. The cpDNA probes constituted a library of cloned *Lactuca sativa* cpDNA fragments (Jansen and Palmer, 1987). The cpDNA probes were maintained as inserts in the plasmid pUC18 that transferred Ampicillin resistance to transformed *Escherichia coli* (see Figure 2.4 for map of location of probes, pLsC1-15, on cpDNA molecule, from Harris, 1990). For

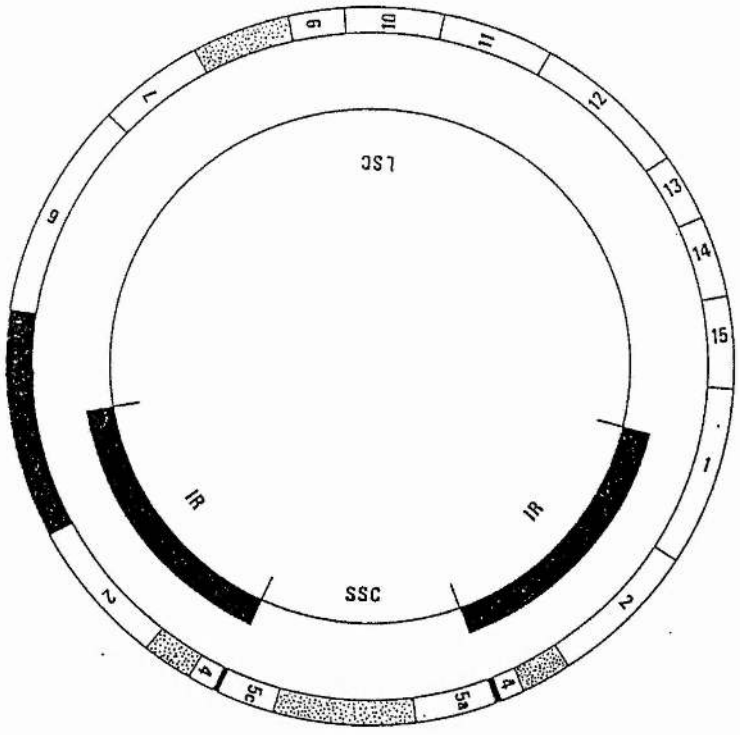


Figure 2.4. Generalized structure of chloroplast DNA (*Lactuca sativa*). The numbers on the outer circle refer to restriction fragments isolated by Jansen and Palmer (1987) and used as probes. SSC - small single copy region, LSC - large single copy, IR - inverted repeat (after Harris, 1990).

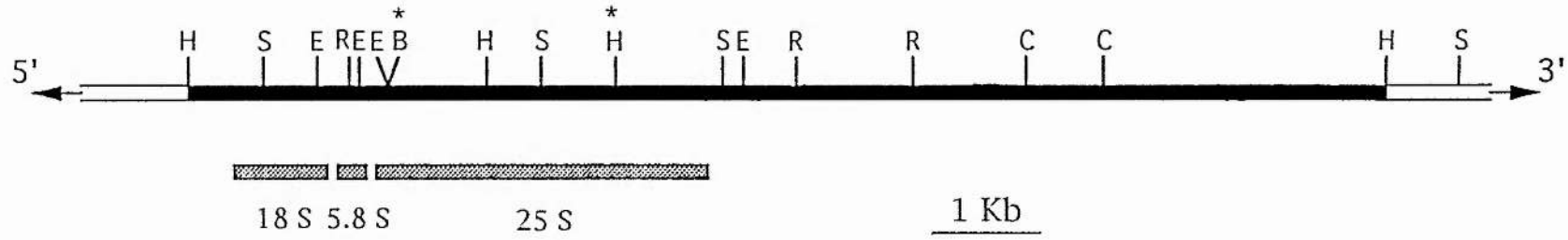


Figure 2.5. Restriction map of rDNA repeat unit of *S. squalidus*. Restriction sites mapped are as follows: *Bgl*II (B), *Bcl*I (C), *Eco*RI (E), *Bam*HI (H), *Eco*RV (R) and *Sac*I (S). * indicates sites at which only partial cleavage occured (after Curnow, unpublished).

Table 2.3. List of *Lactuca sativa* cloned cpDNA and 'Chinease Spring' *Triticum aestivum* rDNA probe inserts, their size and restriction enzyme insert sites.

Probes	Fragment size (Kb)	Restriction enzyme insert site
pLsC1	12.3	*Sac*I
pLsC2	9.9	*Sac*I
pLsC4	1.8	*Sac*I
pLsC5a	5.5	*Hin*DIII
pLsC5c	3.6	*Sac*I-*Hin*DIII
pLsC6	14.7	*Sac*I
pLsC7	7.0	*Sac*I
pLsC9	3.8	*Sac*I
pLsC10	6.9	*Sac*I
pLsC11	7.7	*Sac*I
pLsC12	10.6	*Sac*I
pLsC13	4.6	*Sac*I
pLsC14	5.4	*Sac*I
pLsC15	6.3	*Sac*I
pTa71	9.1	*Eco*RI

rDNA, a single, complete *Triticum aestivum* 'Chinese Spring' rDNA repeat had been cloned into pUC19 (Gerlach and Bedbrook, 1979) and inserts were maintained by transferring Tetracycline resistance to the *E. coli* host (see Figure 2.5 for a restriction map of rDNA repeat unit, probe pTa71, mapped for *S. squalidus* by D. Curnow, unpublished data). *E. coli* hosts were propagated by inoculating nutrient broth cultures (13 g/l Oxoid nutrient broth) containing the appropriate antibiotic (12.5 μg/ml Tetracycline for rDNA probes and 50 μg/ml Ampicillin for cpDNA probes) and incubating overnight at 37° C. Stocks were then frozen and stored at -20° C or centrifuged at 3,000 rpm for 10 minutes. The supernatant was discarded and the bacterial pellet re-suspended in 100 μl of buffered sucrose solution (25% (w/v) Sucrose in 50 ml of 10 mM Tris pH 8.0). The solution was transferred to a 1.5 ml Eppendorf tube and 600 μl MSTET (5% (v/v) Triton, 50 mM EDTA Na_2, 50 mM Tris, 5% (w/v) Sucrose to pH 8.0) were added. Forty μl of freshly prepared lysozyme (40 μg/ml in buffered sucrose solution) were added and the tube was vortexed and placed in boiling water for 1 minute before cooling rapidly on ice. The solution was centrifuged at 13,000 rpm for 45 minutes and the pellet of cellular debris was removed with a sterile toothpick. To the solution 200 μl phenol/0.8% hydroxyquinoline were added and gently mixed to an emulsion before centrifuging again at 13,000 rpm for 10 minutes. From the aqueous layer 600 μl were removed to a new Eppendorf tube and 60 μl of 7.5 M ammonium acetate and up to 1 ml propan-2-ol at -20° C were added before the solution was left at -20° C for at least 1 hour. The solution was centrifuged for 15 minutes at 13,000 rpm before discarding the supernatant and drying the pellet, which was then re-suspended in 100 μl TE. Five μl RNAase (10 μg/μl) were added and the solution was incubated at 37° C for not more than 30 minutes. The plasmid extract was made up to 750 μl with TE and 3 more phenol extractions were done with an equal volume of phenol (i.e. mixing solution, centrifuging for 10 minutes at 13,000 rpm and removing aqueous layer) followed by 2 chloroform (24:1, chloroform:isoamyl alcohol) extractions. After the final extraction, the aqueous layer was transferred to a new Eppendorf tube and 1/10th volume of 7.5 M ammonium acetate was added followed by propan-2-ol at -20° C to the top of the tube. DNA was left to precipitate for 30 minutes and then centrifuged at 13,000 rpm for 30 minutes, before the supernatant was discarded and the pellet left to dry. DNA was re-suspended in 50 μl TE. The concentration of the plasmid DNA was determined by eletrophoresing a portion of the sample through agarose gels with a known concentration marker.

To use probes, inserts were cut from the vector by digestion with the appropriate restriction enzyme (10 units of enzyme for every 1 μg DNA), at the recommended temperature for at least 2 hours. Digestion was then verified by examining the size of

fragments liberated by agarose gel electrophoresis and the concentration of fragments quantified by comparison to a known concentration marker. The digest was de-proteinated by chloroform extraction and probe concentration was standardized to 60 ng DNA/µl and stored at -20° C until needed. The cpDNA and rDNA probes that were used, together with their size and restriction enzyme insertion sites, are listed in Table 2.3.

Radio-labelling of probes

DNA probes were radio-labelled by the random primer extension method of Feinberg and Vogelstein (1983). Labelling quantities depended on the size and quantity of filters used but one 20 x 20 cm filter required 60 ng of probe DNA with 10 µCi α-32P-sCTP. Probe DNA (60 ng) was made up to 13.6 µl with sterile distilled water and denatured by immersion in boiling water for 2-10 minutes followed by quenching on ice. The following were added to the cooled probe; 5 µl of 1 M HEPES pH 6.6, 5 µl DTM (100 mM dATP, 100 mM dGTP, 100 mM dTTP), 1.4 µl OL (1 mM Tris-HCl pH 7.5, 1 mM EDTA Na_2 pH 7.5, 90 optical density units/ml hexaoligodeoxyribonucleotides), 1 µl of 10 µg/ml Bovine serum albumin, 0.5 µl Klenow (5 units/µl) and 1 µl α-32P-dCTP. The probe labelling solution was then incubated at room temperature for 5 hours. Labelled probe was then either used immediately or stored for several days at -20° C.

Pre-hybridization of membranes

Pre-hybridization and hybridization of membranes were carried out in a hybridization oven. The oven was pre-heated to 65° C and if more than one membrane was used, they were laid out in step wise layers in the hybridization tube. 15 ml of pre-hybridization buffer was used per filter up to a maximum of 50 ml per tube. Pre-hybridization buffer consisted of buffer III (0.6 M NaCl, 10 mM PIPES pH 6.8, 1 mM EDTA and 10 x modified Denhardt's solution). Modified Denhardt's solution was kept as 100 x stock solution (2% (w/v) Bovine skin gelatin type B, 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone-360, 10% (w/v) SDS and 0.5% (w/v) tetrasodium pyrophosphate, which was sterilized and kept at 4° C). The pre-hybridization buffer was heated to 60° C and 10 µg/ml of sonicated (600-800 bp), denatured (boiled for 2-10 minutes) calf thymus DNA was added before injecting the solution into the hybridization tubes containing the membrane(s). The membrane(s) were left for at least 6 hours to prehybridize at 65° C with rotation. If more than one membrane was used per tube the direction of tube rotation was reversed after 3 hours.

Cross-hybridization of membranes to probe

Following pre-hybridization of membranes, the radio labelled probe was made up to 1 ml with TE, denatured (held immersed in boiling water for 2-10 minutes) and injected into the hybridization tubes. The membranes were left to hybridize with the labelled probe overnight at 65o C with rotation.

Washing of membranes and auto-radiography

After overnight hybridization, membranes were washed three times in 500 ml 2 x SSC and 0.5% (w/v) SDS for 30 minutes, the first two washes at room temperature and the final wash at 65o C. Membranes were blotted with paper towels before covering with plastic sheeting (to prevent drying) and placing in an auto-radiography cassette. The level of hybridized probe activity was assessed for each membrane using a Geiger-Müller tube and a sheet of X-ray film was placed against the DNA side of each membrane, carefully aligned to the edge of the membrane corresponding to the position of the wells on the original agarose gel. The film was left to expose at -70o C from several hours to 3 weeks, depending on membrane activity. Following exposure the film was developed in an X-ray film processing machine.

Stripping of filters

To permit reprobing of membranes, the radio labelled probes were stripped from the membranes by pouring 500 ml of boiling 0.1% (w/v) SDS onto membranes and leaving them to cool to room temperature. The level of residual probe adherence was assessed with a Geiger-Müller tube and stripping was repeated if necessary. Membranes were then sealed in plastic sheeting and stored at -20o C until used again. Typically membranes could be reprobed up to a maximum of 10 times.

RFLP Fragment size determination

The size of DNA fragments, corresponding to bands on auto-radiographs, were determined by comparing their relative migration distance from the gel well origin to the mobility of *Hin*DIII digested lambda DNA size standards run on the same gels. The sizes of fragments were calculated from these distance measures using an IBM-PC compatible computer program supplied by M. Krawczak (Krawczak, 1988).

Fragment size and mutation type identification for cpDNA and rDNA

The 58 DNA samples examined in the cpDNA RFLP analysis were digested with the restriction enzyme *Cla*I (a 6-bp cutter) and probed with clone pLsC6. This enzyme-probe combination produces a fragment profile that clearly distinguishes individuals that contain an insertion of approximately 330 bp, and further enables differences to be

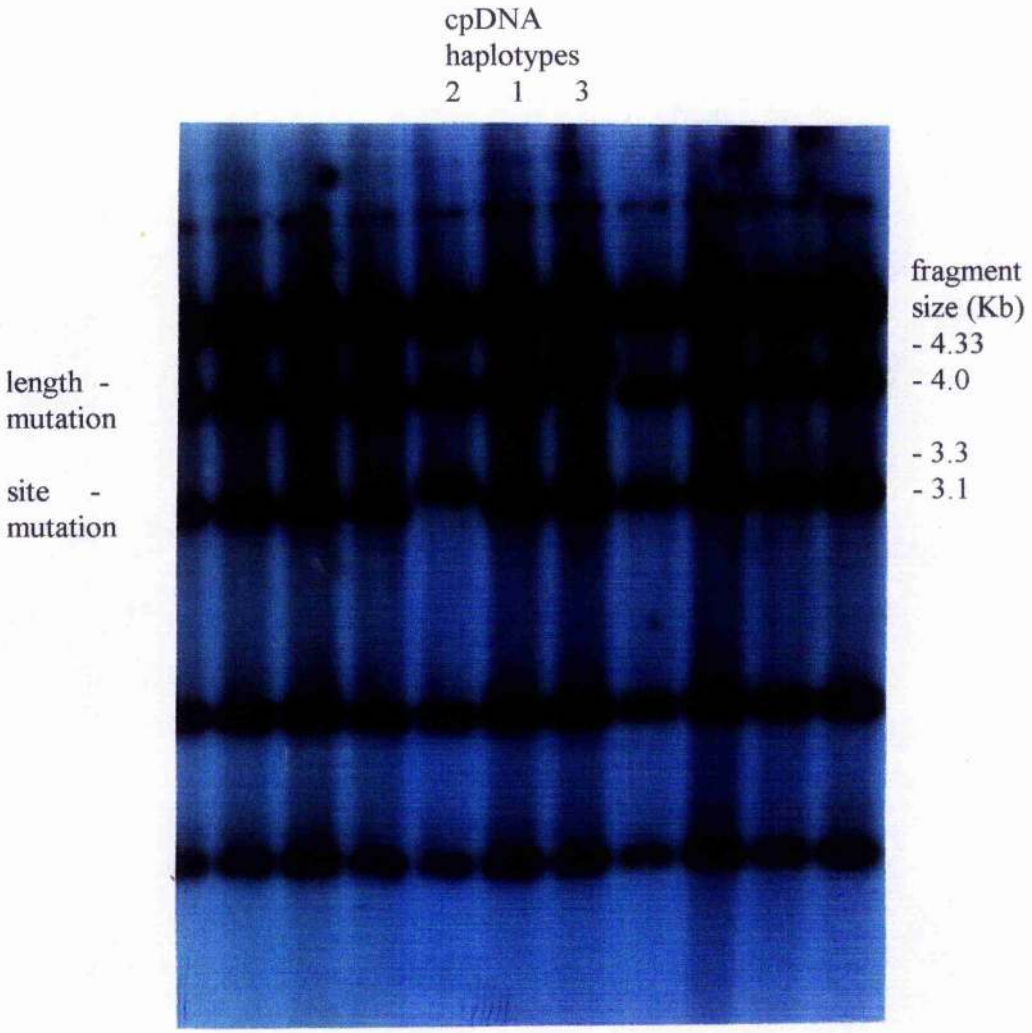


Figure 2.6. Showing an autoradiograph of a sample of total genomic DNA digested with the restriction enzyme *Cla*I and probed with the *Lactuca sativa* cloned cpDNA probe pLsC6. The length and site mutation combinations that constitute the three cpDNA haplotypes are highlighted.

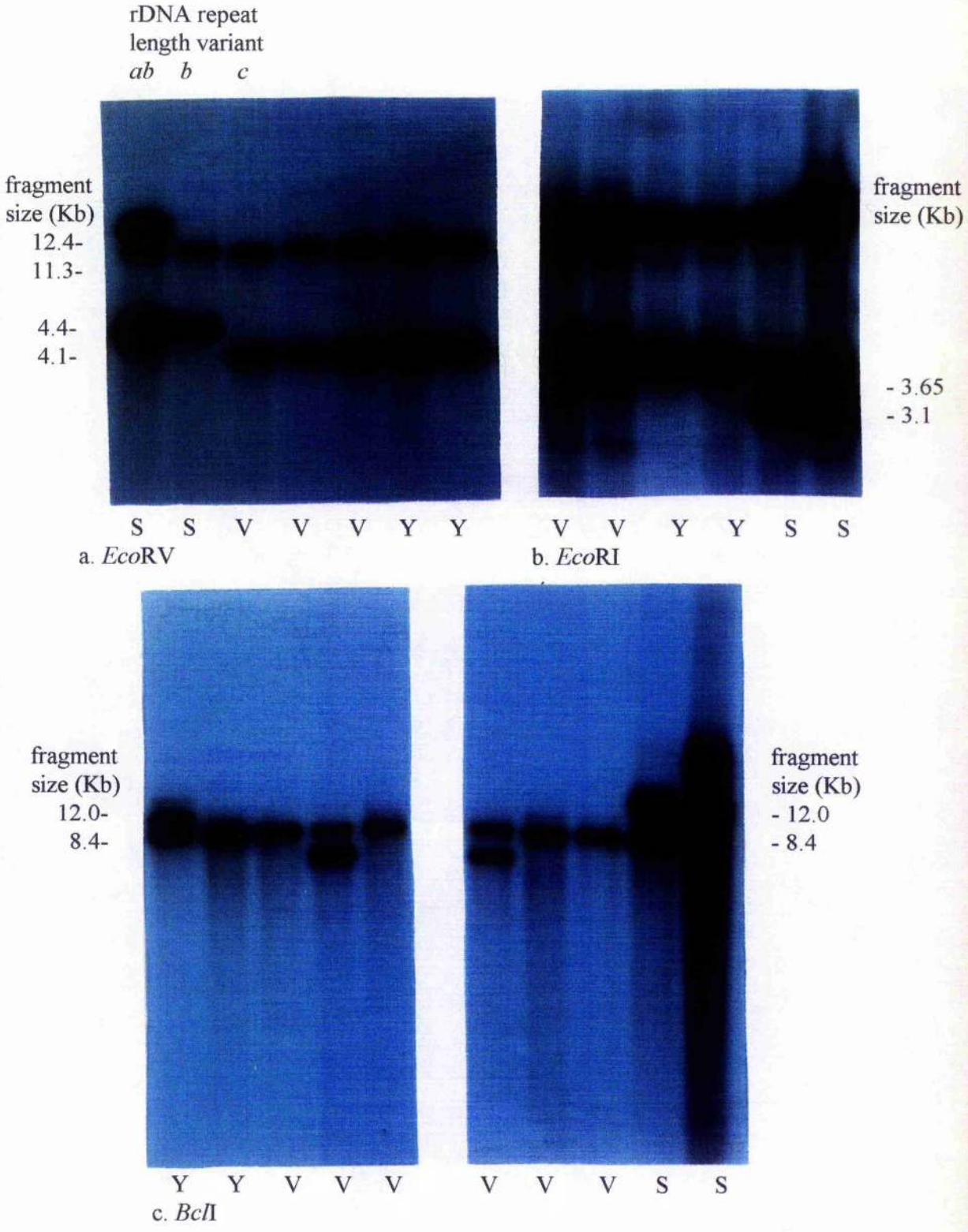


Figure 2.7. Showing autoradiographs of samples of total genomic DNA digested with three restriction enzymes and probed with the 'Chinese Spring' wheat cloned rDNA probe pTa71; a. digested with *Eco*RV; b. digested with *Eco*RI; c. digested with *Bcl*I. The site mutations and rDNA lengths variants that distinguish the taxa are highlighted. Taxa abbreviations; Y, York radiate groundsel; V, *S. vulgaris*; S, *S. squalidus*.

distinguished between individuals at a polymorphic restriction site (Figure 2.6). Three cpDNA haplotypes were defined by the combination of these two mutations. Haplotype 3 possessed the 330 bp insert and restriction site, haplotype 1 lacked the 330 bp insert but possessed the restriction site, while haplotype 2 lacked both the insert and restriction site (Figure 2.6). All DNA samples were identified for one of these three cpDNA haplotypes.

A previous analysis of *S. squalidus* and its related diploid species had identified three different cpDNA haplotypes (Abbott, Curnow and Irwin, 1995). These haplotypes, A, B and C, were distinguished by the same *Cla*I x pLsC6 site mutation and insert combination as haplotypes 1, 2 and 3 in this study, but were also surveyed for six additional enzyme-probe combinations. It is highly likely that haplotypes 1, 2 and 3 represent haplotypes A, B and C respectively. To confirm this, 10 of the 56 DNA samples used in the study, were probed with the extra six enzyme-probe combinations (*Pvu*II x pLsC4&5, *Hae*III x pLsC4&5, *Pst*I x pLsC1-3, *Cfo*I x pLsC6, *Bgl*II x pLsC7 and *Eco*RI x pLsC8-11).

For rDNA RFLP analysis, the 56 DNA extracts were digested with the restriction enzymes *Eco*RI, *Eco*RV and *Bcl*I and probed with the clone pTa71. Previous work by Harris and Ingram (1992a) and D. Curnow (unpublished) showed that these enzyme-probe combinations identify three rDNA restriction site mutations that distinguish *S. vulgaris* from *S. squalidus* (see Figure 2.7). Curnow (unpublished) has further shown that three different rDNA repeat length variants could be resolved among the same two *Senecio* taxa and that the average total length of fragments could be estimated by digests involving *Eco*RI, *Sac*I, *Bgl*II, *Bst*EII and *Bam*HI. Curnow found that the restriction enzyme *Eco*RV produced the clearest differentiation of the repeat length variants, although it cut twice in the IGS region and so cumulative fragment size totals could not be equated to rDNA repeat length sizes. The largest repeat length variant in *Eco*RV digests produced a fragment of 7.2 Kb which identified an rDNA length variant designated as the *a* variant and known to be 12.4 Kb in length. The next longest length variant (11.65 Kb), denoted as *b*, could be detected in the same digests by the presence of a 6.45 Kb fragment, while the shortest length variant (11.3 Kb), denoted as *c*, yielded a 6.1 Kb fragment (see Figure 2.7a). Individuals were scored for presence/absence of the three site mutations and the possession of particular rDNA repeat length variants.

RAPD analysis

RAPD analysis was conducted on eight individuals of *S. vulgaris* var. *vulgaris* from Methil and five from York populations, five *S. vulgaris* var. *hibernicus* individuals from Edinburgh, 11 York radiate groundsel individuals and five individuals of *S. squalidus* from three different populations in the British Isles (see Table 2.1). DNA for RAPD analysis was extracted following the method of Wang, Qi and Cutler (1993). A small piece (1 cm^2) of leaf tissue was quick frozen in liquid nitrogen and ground to a powder in an Eppendorf tube with a micro-pestle. Fifty µl of 0.5 M NaOH were added and the tissue ground further. The extract was centrifuged for 5 minutes at 13,000 rpm and 5 µl of supernatant were removed and added to 495 µl of 100 mM Tris HCl pH 8.0, in a new tube. As this method does not remove DNAases, DNA extracted in this way only remained intact for a maximum of two weeks. Thus DNA was stored at -20° C for a maximum of two weeks before use. Five µl of this solution were used in each PCR reaction and were estimated to contain less than 10 ng DNA.

RAPD-PCR reaction

RAPD-PCR reactions were made up in either 0.5 ml Eppendorf tubes or in 200 µl wells in a 96 (12 x 8) well plate, to which 5 µl DNA solution were added followed by 45 µl of freshly made PCR mega-mix. The quantities of components in the PCR mega-mix were calculated according to the number of RAPD-PCR reactions required, with additional margins for pipetting errors. The volumes and concentrations for one reaction were made up in the following order and briefly vortexed and centrifuged before addition to DNA solution; 31 ml distilled, sterilized water, 5 µl DYNAZYME polymerase buffer (supplied with the enzyme), 4 µl of 25 mM $MgCl_2$, 2.5 µl of 2 mM dNTPs (2 mM dATP, 2 mM dTTp, 2 mM dGTP, 2 mM dCTP), 2 µl of 5 pmol/ml Operon primer (see Table 2.4 for sequence) and 0.5 µl (1 unit) of DYNAZYME *Taq* DNA polymerase. The RAPD-PCR reaction mix was overlaid with 50 µl sterile silicon fluid or mineral oil. Care was taken to prevent contamination with 'foreign' DNA by sterilizing solutions and hardware. The tubes or plates were sealed and placed on a thermocycling machine programmed with the following cyclic temperature profile: an initial step of denaturation at 94° C for 3 minutes, a cyclic denaturation step at 94° C for 30 seconds, a cyclic annealing step at 36° C for 45 seconds with a temperature change rate of 0.4° C/second up to the cyclic extension step at 72° C for 2 minutes. The cyclic steps were repeated 45 times before a final extension step at 72° C for 4 minutes. The samples were then stored at 4° C until fragment profiles were examined by agarose gel electrophoresis.

Table 2.4 List of RAPD primer sequences

RAPD primer	Sequence	RAPD primer	Sequence
OPH01	GGTCGGAGAA	OPA01	CAGGCCCTTC
OPH02	TCGGACGTGA	OPA02	TGCCGAGCTG
OPH03	AGACGTCCAC	OPA03	AGTCAGCCAC
OPH04	GGAAGTCGCC	OPA04	AATCGGGCTG
OPH05	AGTCGTCCCC	OPA05	AGGGGTCTTG
OPH06	ACGCATCGCA	OPA06	GGTCCCTGAC
OPH07	CTGCATCGTG	OPA07	GAAACGGGTG
OPH08	GAAACACCCC	OPA08	GTGACGTAGG
OPH09	TGTAGCTGGG	OPA09	GGGTAACGCC
OPH10	CCTACGTCAG	OPA10	GTGATCGCAG
OPH11	CTTCCGCAGT	OPA11	CAATCGCCGT
OPH12	ACGCGCATGT	OPA12	TCGGCGATAG
OPH13	GACGCCACAC	OPA13	CAGCACCCAC
OPH14	ACCAGGTTGG	OPA14	TCTGTGCTGG
OPH15	AATGGCGCAG	OPA15	TTCCGAACCC
OPH16	TCTCAGCTGG	OPA16	AGCCAGCGAA
OPH17	CACTCTCCTC	OPA17	GACCGCTTGT
OPH18	GAATCGGCCA	OPA18	AGGTGACCGT
OPH19	CTGACCAGCC	OPA19	CAAACGTCGG
OPH20	GGGAGACATC	OPA20	GTTGCGATCC

The melting point of primers can be calculated by the formula (4 x (G + C)) + (2 x (A + T)), but for RAPD-PCR decamer primers which have a G/C content generally greater than 60%, the melting point was assumed to be 37o C.

Gel electrophoresis

A 1.4% (w/v) agarose gel was made up in 300 ml of 0.5 x TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0). The post-PCR reaction solutions were mixed with 1/10th volume (5 μl) gel loading buffer and loaded into wells. A size standard (123 bp ladder) was also loaded into the two outer lanes of each gel to allow the sizing of fragments. Gels were run at 90 mA for four hours and then examined under transmitted UV light and photographed.

RAPD Fragment size determination

RAPD fragment sizes were determined by measuring the distance of migration of each band relative to the migration of the fragments of the size standard. No program was available to estimate fragment sizes from distance measures relative to a 123 bp ladder, but the size standard fragments were sufficiently regular to allow sizing of fragments by eye to within an acceptable margin of error.

Analysis of presence or absence of RAPD fragments

The presence or absence of each RAPD fragment was scored for all individuals examined and a matrix was constructed. Only reliably amplified fragments were scored. Similarity measures were calculated according to Gower (1971) by the program PCO3D (Adams, 1995), taking both the presence and absence of fragments as indicating genetic similarity. The shared absence of RAPD fragments can accurately indicate genetic similarity in closely related individuals as there is a high probability that the absence of a fragment is due to ancestry rather than convergent evolution (Adams, 1995). Principal coordinate analysis (PCO) from the genetic similarity matrix followed Gower (1966). Eigenroots were computed by the Householder-Oretoga-Wilkenson method (see Cooley and Lohnes, 1971) and coordinate axes were assessed for significance by comparison to the average value of the diagonal elements. The robustness of clusters defined by PCO were examined by comparison of the minimum similarity between individuals within a cluster to the average similarity between all individuals. PCO has advantages over other multivariate statistical methods in the analysis of RAPD data. Principal component analysis (PCA) and canonical variate analysis (CVA) are strongly affected by the non-normal data distribution and non-continuous discrete presence and absence data of RAPD fragments, which is not the case for PCO. Graphical plotting of PCO eigen values was

chosen in preference to a dendrogram building algorithm, as it overrides the problems associated with possible ambiguous resolution of hybrids in dendrograms (Rieseberg and Ellstrand, 1993).

Results

Morphometric analysis, first study

Single character analysis

From the results of a one-way ANOVA of each character taken in turn (Table 2.5), it was clear that York radiate groundsel was intermediate in mean phenotype between *S. vulgaris* var. *vulgaris* and *S. squalidus* for eight characters measured in the first morphological analysis (C2, inflorescence length; C5, capitulum width; C8, number of calyculous bracts; C10, number of ray florets; C11, mean outer floret length; C12, mean outer floret width; C17, midleaf secondary vein angle; C22, total number of seeds per capitulum). For one character (C16, midleaf apical angle), York radiate groundsel was more similar to *S. squalidus* than *S. vulgaris*, whereas for six characters (C1, plant height; C6, number of phyllaries; C13, longest leaf length; C18, leaf dissection; C20, standardized square of leaf area; C25, proportion of self seed set), it was more similar to *S. vulgaris*. York radiate groundsel possessed seven novel characters (i.e. characters with a mean lying outside the range of variation that spanned *S. vulgaris* and *S. squalidus*). These were long, many lobed leaves (C14, C15, C19), long achenes (C21), long calyculus bracts (C9), a low proportion of black tipped phyllaries (C7) and four pored pollen (C23). In contrast to York radiate groundsel, *S. vulgaris* var. *hibernicus* from Edinburgh, was intermediate between *S. vulgaris* var. *vulgaris* and *S. squalidus* in mean phenotype for only four characters (C10, number of ray florets; C11, mean outer floret length; C12, mean outer floret width; C14, midleaf length) and was not significantly different from var. *vulgaris* for the remaining 22 characters examined.

Multivariate analysis

Canonical variate analysis of the first morphological data set revealed that the first two canonical variables were statistically significant ($P < 0.001$), and accounted for 81% and 11% of the total variance in measurements respectively. The total-sample standardized canonical coefficients (SCC) are listed in Table 2.6, and characters with values greater than 1.0 were presumed to make a significant contribution to a particular canonical axis. Consequently plants with high values for the first canonical variable had longer and wider outer (ray) florets (C11, C12), and more highly dissected, smaller leaves (C18, C19, C20), while plants with higher values for the second canonical variable were short (C1) and had long and narrow ray florets (C11, C12), fewer, longer seeds (C21, C22), a low proportion of phyllaries with black tips (C7) and a longer, more lobed midleaf (C15, C19, C20).

Table 2.5. Means, significant differences (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns-not significant) and results of Tukey-Kramer multiple comparison for 26 morphological traits measured on York radiate groundsel and Edinburgh and York individuals of *S. squalidus*, *S. vulgaris* var. *vulgaris* and Edinburgh var. *hibernicus*, in the first morphometric analysis. Means sharing the same superscript are not significantly different ($P \leq 0.05$). Standard deviation are shown in parentheses.

Taxon	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *hibernicus*	York radiate groundsel	*S. squalidus*	*S. squalidus*	
Location	York	Edinburgh	Edinburgh	York	Edinburgh	York	
Sample size	n=10	n=6	n=12	n=10	n=9	n=10	
Character							*P*
C1 Plant height[f](cm)	26.51 (2.83)^{bc}	21.14 (2.19)^{d}	23.32 (3.22)^{cd}	20.56 (3.77)^{d}	32.22 (6.49)^{b}	49.47 (10.61)^{a}	***
C2 Inflorescence length[f](mm)	18.33 (2.81)^{b}	19.03 (2.85)^{ab}	17.27 (2.23)^{b}	22.15 (5.15)^{ab}	19.77 (2.16)^{ab}	25.09 (4.37)^{a}	***
C3 Pedicel length[f](mm)	8.18 (2.51)^{ab}	8.82 (2.71)^{ab}	7.72 (1.86)^{b}	11.83 (4.82)^{ab}	9.47 (3.73)^{ab}	14.64 (4.82)^{a}	*
C4 Capitulum length[f](mm)	10.15 (0.69)	10.21 (1.14)	9.56 (0.91)	10.32 (0.72)	10.30 (2.52)	10.44 (0.67)	ns
C5 Capitulum width[f](mm)	3.49 (0.13)^{c}	3.55 (0.27)^{bc}	3.82 (0.41)^{bc}	3.98 (0.19)^{b}	5.24 (0.32)^{a}	4.94 (0.48)^{a}	***
C6 Number of phyllaries	20.30 (0.67)^{ab}	19.17 (0.98)^{ab}	19.25 (2.09)^{ab}	18.20 (1.75)^{b}	21.44 (1.51)^{a}	22.60 (2.32)^{a}	***
C7 Proportion of phyllaries with black tips[g]	0.98 (0.02)^{ab}	0.98 (0.04)^{ab}	0.91 (0.09)^{b}	0.27 (0.23)^{c}	0.94 (0.11)^{ab}	1.00 (0.00)^{a}	***
C8 Number of calyculus bracts	10.90 (1.59)^{a}	10.50 (1.22)^{ab}	11.67 (3.89)^{a}	6.80 (1.48)^{bc}	6.11 (1.05)^{c}	7.30 (1.64)^{c}	***
C9 Mean calyculus bract length[f](mm)	2.98 (0.44)^{b}	3.01 (0.49)^{b}	3.33 (0.63)^{b}	3.96 (0.24)^{a}	3.03 (0.22)^{b}	2.83 (0.39)^{b}	***
C10 Number of ray florets	0.00 (0.00)^{d}	0.00 (0.00)^{d}	11.67 (1.37)^{b}	8.70 (0.95)^{c}	12.56 (0.73)^{ab}	13.00 (0.00)^{a}	***
C11 Mean outer floret length (mm)	5.00 (0.00)^{c}	5.00 (0.00)^{c}	17.47 (0.69)^{b}	16.51 (0.52)^{b}	34.32 (3.63)^{a}	33.50 (4.44)^{a}	***
C12 Mean outer floret width (mm)	1.00 (0.00)^{d}	1.00 (0.00)^{d}	1.16 (0.01)^{c}	1.15 (0.01)^{c}	1.44 (0.07)^{a}	1.37 (0.06)^{b}	***
C13 Longest leaf length[f](cm)	15.78 (1.57)^{ab}	11.93 (2.98)^{c}	16.17 (1.82)^{ab}	18.18 (1.98)^{a}	13.19 (1.44)^{bc}	14.46 (2.91)^{bc}	***
C14 Midleaf length[f](cm)	11.14 (0.61)^{ab}	9.81 (3.06)^{bcd}	10.32 (2.03)^{bc}	13.70 (2.42)^{a}	7.49 (1.79)^{d}	8.17 (1.59)^{cd}	***
C15 Number of midleaf lobes	12.30 (0.67)^{b}	11.33 (1.03)^{b}	11.92 (0.90)^{b}	16.80 (1.23)^{a}	10.89 (1.45)^{b}	11.40 (1.89)^{b}	***
C16 Midleaf apical angle (degrees)	139.40 (17.29)^{a}	132.00 (8.84)^{a}	137.88 (18.60)^{a}	100.60 (14.09)^{b}	85.56 (14.56)^{b}	88.15 (14.26)^{b}	***
C17 Midleaf secondary vein angle (degrees)	59.50 (9.58)^{ab}	67.83 (6.71)^{a}	66.21 (7.28)^{a}	57.85 (8.50)^{ab}	49.67 (7.05)^{bc}	45.55 (11.69)^{c}	***
C18 Leaf dissection[h]	2.58 (0.56)^{c}	3.32 (1.39)^{bc}	3.97 (1.32)^{bc}	3.71 (0.50)^{bc}	9.31 (5.33)^{a}	6.79 (1.46)^{ab}	***
C19 Standardized leaf perimeter[i]	21.39 (2.54)^{b}	19.59 (8.27)^{b}	26.48 (6.74)^{b}	37.00 (5.52)^{a}	23.05 (8.50)^{b}	24.91 (7.64)^{b}	***
C20 Standardized square of leaf area[i]	1.87 (0.19)^{a}	1.68 (0.33)^{ab}	1.73 (0.21)^{a}	1.97 (0.22)^{a}	1.22 (0.42)^{c}	1.33 (0.19)^{bc}	***
C21 Seed length[f](mm)	2.18 (0.15)^{c}	2.39 (0.26)^{bc}	2.45 (0.25)^{b}	2.95 (0.22)^{a}	2.26 (0.13)^{bc}	2.35 (0.05)^{bc}	***
C22 Total number of seeds per capitulum	68.20 (4.21)^{b}	56.50 (10.67)^{bc}	52.42 (10.21)^{c}	63.80 (5.73)^{b}	83.22 (11.90)^{a}	90.90 (7.32)^{a}	***
C23 Number of pollen pores[f]	3.00 (0.00)^{b}	3.00 (0.00)^{b}	3.00 (0.00)^{b}	4.00 (0.00)^{a}	3.00 (0.00)^{b}	3.00 (0.00)^{b}	***
C24 Pollen pore size[f] (graticule units 40=0.1mm)	8.15 (1.29)^{ab}	8.33 (1.51)^{ab}	8.83 (1.40)^{a}	7.15 (1.25)^{b}	7.99 (0.63)^{ab}	8.9 (1.37)^{a}	***
C25 Proportion of self seed set per capitulum[g]	0.74 (0.14)^{a}	0.79 (0.12)^{a}	0.77 (0.18)^{a}	0.86 (0.12)^{a}	0.06 (0.13)^{b}	0.00 (0.00)^{b}	***
C26 Time to apical capitulum anthesis[f] (days)	73.10 (6.87)	76.00 (8.67)	70.83 (5.64)	71.20 (3.29)	96.44 (10.22)	92.50 (30.43)	ns

[f] Log[e] transformed.
[g] Arcsine transformed.
[h] Calculated as midleaf perimeter divided by the square root of area; high ratio indicating highly divided leaf.
[i] Perimeter and square of area measures were divided by midleaf length to standardize.

Table 2.6. Total-sample standardized canonical coefficients for the 22 morphometric characters with respect to canonical variate 1 and 2.

Character	CV1	CV2
C1 Plant height	0.933	-1.544
C2 Inflorescence length	0.151	0.007
C3 Pedicel length	-0.095	-0.088
C5 Capitulum width	0.642	0.507
C6 Number of phyllaries	-0.409	-0.417
C7 Proportion of phyllaries with black tips	-0.383	-1.657
C8 Number of calyculus bracts	-0.689	-0.096
C9 Mean calyculus bract length	-0.399	0.031
C11 Mean outer floret length	5.475	1.568
C12 Mean outer floret width	1.604	-1.376
C13 Longest leaf length	-0.168	-0.650
C14 Midleaf length	-0.239	0.722
C15 Number of midleaf lobes	0.353	1.326
C16 Midleaf apical angle	-0.384	-0.549
C17 Midleaf secondary vein angle	-0.449	0.067
C18 Midleaf dissection	1.015	0.313
C19 Standardized leaf perimeter	1.479	2.093
C20 Standardized square of leaf area	-1.053	-1.129
C21 Mean seed length	0.269	1.111
C22 Total number of seeds	0.313	-1.056
C24 Pollen pore size	-0.562	-0.683
C25 Proportion of self seed set	-0.496	-0.338

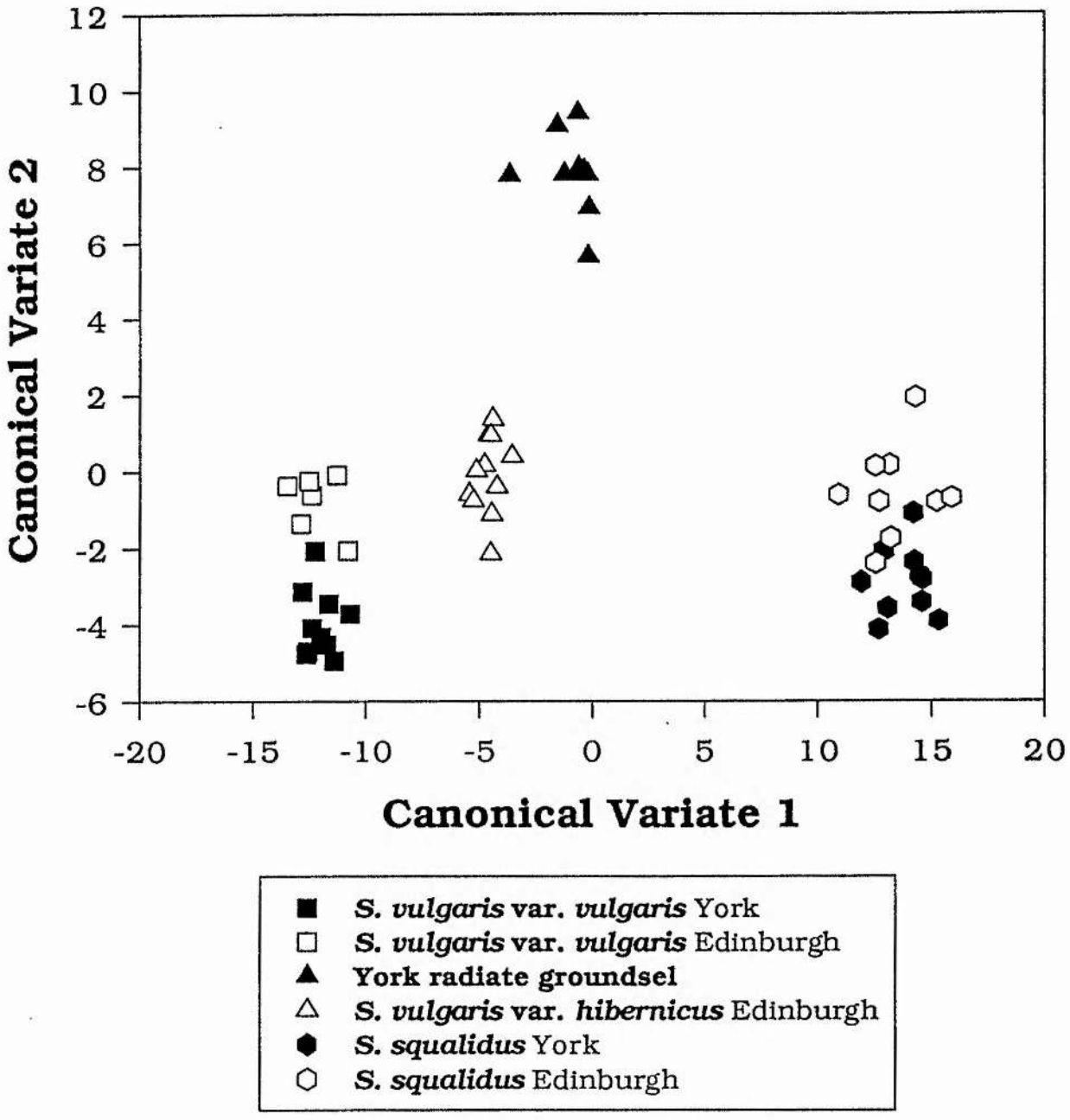


Figure 2.8. Results of canonical variate analysis of the first morphometric data set (m1). Each point represents the scores (CV1 vs CV2) for an individual within a given taxon/population.

Table 2.7. Mahalanobis' distances between taxa (level of significance indicated in parentheses).

	S. vulgaris var. *vulgaris* Edinburgh	*S. squalidus* Edinburgh	*S. vulgaris* var. *vulgaris* York	York radiate groundsel	*S.squalidus* York
S. vulgaris var. *hibernicus* Edinburgh	101.89 (***)	351.91 (***)	127.99 (***)	120.44 (***)	338.96 (***)
S. vulgaris var. *vulgaris* Edinburgh		666.72 (***)	32.40 (**)	216.56 (***)	698.30 (***)
S. squalidus Edinburgh			676.10 (***)	290.57 (***)	29.62 (***)
S. vulgaris var. *vulgaris* York				263.48 (***)	673.86 (***)
York radiate groundsel					334.02 (***)

** $P \leq 0.01$
*** $P \leq 0.001$

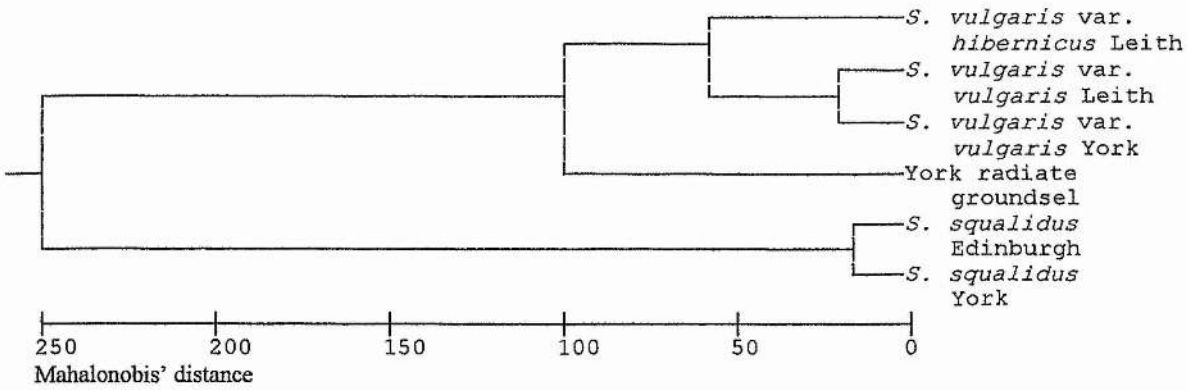


Figure 2.9. Dendrogram produced by UPGMA plot of Mahalonobis' distances calculated from morphometric character measurements.

Canonical variable 1 separated plants of *S. vulgaris* var. *vulgaris* and *S. squalidus* into two distinct groups (Figure 2.8) and placed the York radiate groundsel intermediate between these two groupings. In contrast, *S. vulgaris* var. *hibernicus* plants from Edinburgh were placed close to *S. vulgaris* var. *vulgaris* material. Canonical variable 2 separated York radiate groundsel plants from its parental taxa and *S. vulgaris* var. *hibernicus*, due mainly to the high weighting on this axis of the novel characters exhibited by York plants.

Examination of Mahalanobis' distances revealed that all taxonomic units were significantly differentiated from each other (Table 2.7). The phenogram produced by UPGMA from these distances (Figure 2.9) showed that whilst York radiate groundsel is intermediate between *S. squalidus* and *S. vulgaris*, it is more similar in morphology to the *S. vulgaris* grouping. This analysis again showed that York radiate groundsel is clearly distinct in morphology from *S. vulgaris* var. *hibernicus*.

Morphometric analysis, second study

Single character analysis

Single character analysis of floral characters recorded in the second morphological study, together with the linear and angular measurements derived from leaf landmark coordinates, again showed that York radiate groundsel tended to exhibit a phenotype intermediate to *S. vulgaris* var. *vulgaris* and *S. squalidus*; but also possessed some novel characters which, in mean expression, lay outside the range of variation that spanned the two putative parents (Table 2.8). York radiate groundsel plants were intermediate between *S. vulgaris* var. *vulgaris* and *S. squalidus* for seven characters (C2, inflorescence length; C3, peduncle length; C4 capitulum length; C10, number of ray florets; C11, mean outer floret length; C28, total number of pollen grains; C29, number of stigmatic papillae); were more similar to *S. squalidus* than *S. vulgaris* for two characters (C38, basal auricle extension beyond stem attachment; C44, secondary vein angle of apical adjacent lobe), and were more similar to var. *vulgaris* than *S. squalidus* for ten characters (C1, plant height; C9, mean calyculous bract length; C19, standardized leaf perimeter; C22, total number of seeds per capitulum; C26, time to apical capitulum anthesis; C27, number of peduncle bracts; C36, mean basal auricle height; C37, basal lamina width; C41, width of two apical lobes; C45, mid-lobe secondary vein angle). For ten characters, York radiate groundsel exhibited a novel phenotype (C6, number of phyllaries; C8, number of calyculous bracts; C15, number of midleaf lobes; C18, midleaf dissection; C21, mean seed length; C23, number of pollen pores; C30, midleaf length; C34, mid-lobe lamina width; C35, basal auricle width; C46, mid-lobe apical angle). In contrast, *S. vulgaris* var. *hibernicus* (from

Table 2.8. Means, standard deviations, significant differences (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns-not significant) and results of Tukey-Kramer multiple comparison for 39 morphological traits measured on York individuals of *S. squalidus*, *S. vulgaris* var. *vulgaris* and York radiate groundsel, and Methil individuals of var. *vulgaris* and Edinburgh individulas of var. *hibernicus*, in the second morphometric analysis. Means sharing the same superscript are not significantly different ($P \leq 0.05$), and standard deviations are shown in parentheses.

Taxon	*S. vulgaris* var. *vulgaris*		*S. vulgaris* var. *vulgaris*		*S. vulgaris* var. *hibernicus*		York radiate groundsel		*S. squalidus*		
Location	Methil		York		Edinburgh				York		
Sample size	n=19		n=14		n=5		n=40		n=19		
Character											*P*
C1 Plant height (cm)	24.89	$(7.56)^{b}$	18.20	$(4.13)^{c}$	23.54	$(4.92)^{bc}$	21.46	$(3.13)^{bc}$	41.93	$(4.11)^{a}$	***
C2 Infloresence length[f] (cm)	1.22	$(0.19)^{c}$	1.11	$(0.31)^{c}$	1.85	$(0.66)^{b}$	1.78	$(0.47)^{b}$	3.08	$(0.74)^{a}$	***
C3 Pedicel length[f] (cm)	0.511	$(0.15)^{bc}$	0.364	$(0.36)^{c}$	1.096	$(0.65)^{b}$	0.972	$(0.46)^{b}$	2.174	$(0.71)^{a}$	***
C4 Capitulum length (cm)	0.710	$(0.10)^{c}$	0.745	$(0.07)^{c}$	0.756	$(0.03)^{bc}$	0.808	$(0.06)^{b}$	0.907	$(0.06)^{a}$	***
C6 Number of phyllaries	18.89	$(2.05)^{c}$	20.93	$(1.21)^{b}$	20.80	$(1.30)^{bc}$	15.30	$(1.62)^{d}$	24.00	$(3.25)^{a}$	***
C8 Number of calyculus bracts	10.84	$(1.95)^{ab}$	9.79	$(1.42)^{bc}$	13.00	$(2.55)^{a}$	5.25	$(1.35)^{d}$	8.42	$(1.64)^{c}$	***
C9 Mean calyculus bract length (mm)	0.253	$(0.16)^{c}$	0.346	$(0.03)^{ab}$	0.299	$(0.02)^{bc}$	0.395	$(0.03)^{a}$	0.301	$(0.03)^{b}$	***
C10 Number of ray florets	0.00	$(0.00)^{c}$	0.00	$(0.00)^{c}$	8.00	$(2.81)^{b}$	7.96	$(0.86)^{b}$	12.49	$(0.54)^{a}$	***
C11 Mean outer floret length[f] (mm)	0.183	$(0.02)^{c}$	0.189	$(0.01)^{c}$	0.397	$(0.02)^{b}$	0.422	$(0.05)^{b}$	1.045	$(0.12)^{a}$	***
C15 Number of midleaf lobes	10.95	$(1.13)^{b}$	10.43	$(0.85)^{b}$	11.40	$(1.14)^{b}$	15.95	$(1.66)^{a}$	10.16	$(1.43)^{b}$	***
C18 Leaf dissection[g]	12.69	$(2.31)^{c}$	13.62	$(1.19)^{c}$	11.00	$(1.07)^{c}$	18.94	$(2.98)^{a}$	16.56	$(3.48)^{b}$	***
C19 Standardised leaf perimeter[h]	1.121	$(0.29)^{c}$	1.463	$(0.21)^{ab}$	1.135	$(0.30)^{bc}$	1.529	$(0.29)^{a}$	1.269	$(0.31)^{bc}$	***
C20 Standardised square of leaf area[h]	4.41	$(0.98)^{b}$	5.29	$(0.78)^{ab}$	3.98	$(0.39)^{b}$	6.00	$(1.28)^{a}$	5.86	$(1.31)^{a}$	***
C21 Mean seed length (mm)	0.223	$(0.01)^{c}$	0.235	$(0.01)^{bc}$	0.228	$(0.01)^{bc}$	0.293	$(0.01)^{a}$	0.238	$(0.02)^{b}$	***
C22 Total number of seeds per capitulum[f]	16.83	$(2.43)^{c}$	26.75	$(9.32)^{b}$	29.87	$(19.52)^{bc}$	28.90	$(11.45)^{b}$	91.99	$(11.34)^{a}$	***
C23 Number of pollen pores	2.96	$(0.13)^{b}$	3.00	$(0.00)^{b}$	3.00	$(0.00)^{b}$	4.00	$(0.00)^{a}$	3.00	$(0.00)^{b}$	***
C26 Time to apical capitulum anthesis[f] (days)	36.68	$(7.01)^{b}$	32.86	$(2.54)^{b}$	39.00	$(10.86)^{b}$	36.30	$(7.21)^{b}$	63.58	$(12.97)^{a}$	***
C27 Number of pedicel bracts[f]	1.632	$(0.49)^{b}$	1.071	$(0.27)^{c}$	1.600	$(0.55)^{bc}$	1.100	$(0.30)^{c}$	4.789	$(2.68)^{a}$	***
C28 Total number of pollen grains[f]	270.4	$(79.1)^{d}$	273.6	$(68.3)^{d}$	451.2	$(124.2)^{c}$	632.0	$(124.4)^{b}$	2876.0	$(416.0)^{a}$	***
C29 Number of stigmatic papillae[f]	5.63	$(2.29)^{c}$	6.79	$(2.61)^{c}$	5.40	$(1.95)^{c}$	21.08	$(2.49)^{b}$	47.21	$(9.19)^{a}$	***
C30 Midleaf length (cm)	9.30	$(1.58)^{b}$	9.77	$(0.91)^{b}$	8.61	$(2.03)^{b}$	15.33	$(1.46)^{a}$	9.95	$(1.26)^{b}$	***
C31 Apical lobe length (cm)	2.29	$(0.75)^{b}$	2.70	$(0.49)^{ab}$	2.29	$(0.74)^{b}$	3.30	$(0.74)^{a}$	2.89	$(0.83)^{ab}$	***
C32 Apical lobe basal width[f] (cm)	0.608	$(0.19)^{b}$	0.775	$(0.14)^{ab}$	0.700	$(0.17)^{ab}$	0.780	$(0.17)^{a}$	0.724	$(0.22)^{ab}$	*
C33 Mid-lobe vein length[f] (cm)	1.91	$(0.43)^{c}$	2.67	$(0.63)^{b}$	1.71	$(0.52)^{c}$	2.84	$(0.72)^{b}$	3.47	$(0.74)^{a}$	***
C34 Mid-lobe lamina width (cm)	0.363	$(0.13)^{b}$	0.343	$(0.06)^{b}$	0.280	$(0.05)^{b}$	0.476	$(0.10)^{a}$	0.350	$(0.22)^{b}$	***
C35 Basal auricle width (cm)	1.11	$(0.39)^{c}$	1.62	$(0.45)^{b}$	0.93	$(0.13)^{c}$	2.18	$(0.54)^{a}$	0.89	$(0.40)^{c}$	***
C36 Mean basal auricle height (cm)	1.57	$(0.39)^{bc}$	2.09	$(0.49)^{a}$	1.33	$(0.31)^{bc}$	1.85	$(0.54)^{ab}$	1.33	$(0.41)^{c}$	***
C37 Basal lamina width[f] (cm)	0.387	$(0.11)^{bc}$	0.582	$(0.14)^{a}$	0.390	$(0.02)^{abc}$	0.504	$(0.19)^{ab}$	0.266	$(0.13)^{c}$	***
C38 Basal auricle extension beyond stem attachment[f] (mm)	1.79	$(0.66)^{bc}$	1.26	$(0.29)^{a}$	1.42	$(0.32)^{ab}$	2.06	$(0.33)^{c}$	1.87	$(0.53)^{bc}$	***
C39 Right basal lobe width (cm)	0.98	$(0.41)^{b}$	0.64	$(0.45)^{a}$	1.11	$(0.52)^{ab}$	1.66	$(0.47)^{a}$	1.75	$(0.79)^{a}$	***
C40 Height of left basal lobe (cm)	2.86	(0.92)	3.36	(1.20)	2.98	(1.31)	3.29	(1.01)	3.54	(1.06)	ns
C41 Width of two apical lobes	2.33	$(0.56)^{bc}$	2.92	$(0.34)^{a}$	2.22	$(0.54)^{abc}$	2.73	$(0.65)^{ab}$	2.19	$(0.59)^{c}$	***
C42 Apical angle A	119.60	$(35.38)^{a}$	113.70	$(26.71)^{ab}$	123.57	$(15.79)^{ab}$	95.82	$(14.27)^{b}$	96.68	$(21.71)^{b}$	***
C43 Apical angle B	90.45	(19.57)	98.07	(10.77)	91.99	(13.80)	92.78	(15.82)	92.48	(20.15)	ns
C44 Secondary vein angle of apical adjacent lobe	44.55	$(9.23)^{a}$	43.29	$(7.49)^{a}$	36.23	$(3.24)^{ab}$	33.72	$(8.13)^{b}$	29.15	$(7.27)^{b}$	***
C45 Mid-lobe secondary vein angle	70.43	$(7.31)^{ab}$	63.08	$(10.23)^{b}$	66.92	$(10.90)^{ab}$	73.98	$(8.78)^{a}$	48.73	$(7.74)^{c}$	***
C46 Mid-lobe apical angle	119.7	$(44.4)^{a}$	109.5	$(58.6)^{a}$	97.1	$(47.5)^{ab}$	58.7	$(15.78)^{b}$	95.6	$(60.9)^{a}$	***
C47 Basal angle A	107.0	(19.78)	94.9	(26.96)	100.6	(11.28)	102.4	(27.59)	109.0	(22.81)	***
C48 Basal angle B	39.65	$(10.52)^{b}$	54.64	$(9.95)^{a}$	39.96	$(5.12)^{ab}$	54.96	$(13.06)^{a}$	54.12	$(15.18)^{a}$	***

[f] Log^{e} transformed.

[g] Calculated as midleaf perimeter divided by the square root of area; high ratio indicating highly divided leaf.

[h] Perimeter and square of area measures were divided by midleaf length to standardize.

Edinburgh), was intermediate between var. *vulgaris* and *S. squalidus* for six characters (C2, inflorescence length; C3, peduncle length; C10, number of ray florets; C11, mean outer floret length; C28 total number of pollen grains; C44, secondary vein angle of apical adjacent lobe), and was not significantly different from var. *vulgaris* for all other characters measured.

Multivariate analysis

Canonical variate analysis of all morphometric characters showed that the first two canonical variables were statistically significant ($P < 0.001$), and accounted for 62.7% and 33.8% of the total variance in measurements respectively. Plants with high values for the first canonical variable were tall (C1) and had long inflorescences (C2), short capitula (C4) and many ray florets (C10). Individuals with higher values for the second canonical variable had short and less lobed midleaves (C15, C30), and short inflorescences (C2) with many ray florets (C10).

Canonical variable 1 separated *S. vulgaris* var. *vulgaris* and *S. squalidus* plants into two distinct groups (Figure 2.10) and placed York radiate groundsel individuals intermediate between these two groupings and distinct from var. *hibernicus* plants from Edinburgh. The distributions were very similar to those observed in the first morphometric study (see Figure 2.6).

The morphometric data set was then split into two subsets, with one subset of data comprising 16 characters mainly derived from capitulum measurements and the other comprising 23 leaf characters. These two data sets were analysed separately by canonical variate analysis to determine the relative influence of different character suites on the multivariate separation of taxa.

Multivariate analysis of floral character subset

Canonical variable one separated *S. vulgaris* from *S. squalidus* and placed York radiate groundsel intermediate between the two parental taxa (Figure 2.11). The separation of taxa based on capitulum characters was very similar to that observed for the entire data set (Figure 2.10) indicating the disproportionate affect that the number and length of ray florets have in separating these groups.

Multivariate analysis of leaf character subset

Canonical variable one separated York radiate groundsel individuals from all other taxa mainly due to their possession of novel leaf characters, i.e. typically long and many lobed leaves (Figure 2.12). Canonical variable two separated *S. squalidus* from

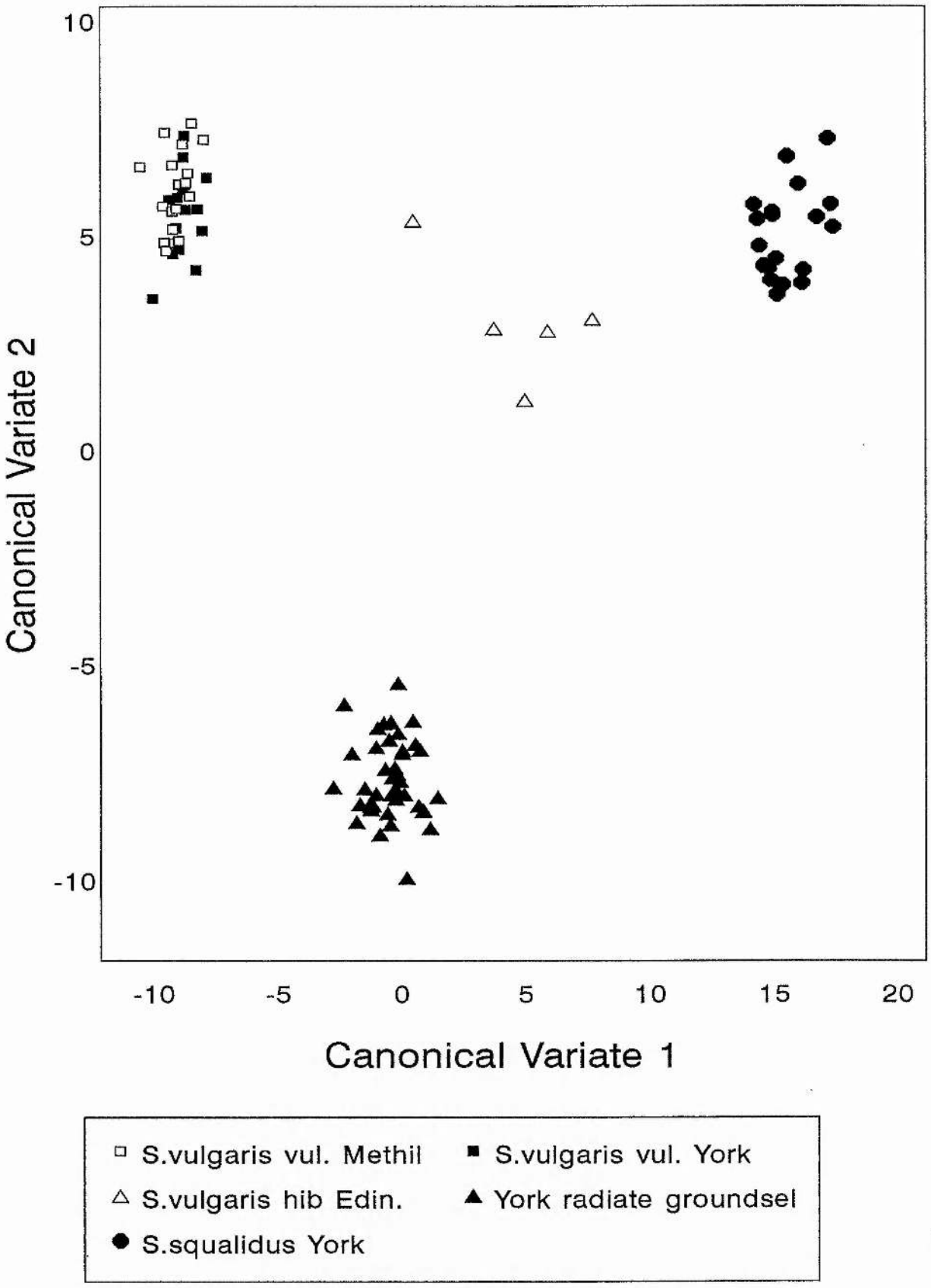


Figure 2.10. A plot of the scores (CV1 vs CV2) for each individual within a given taxon/population following canonical variate analysis of the entire data set obtained from the second morphometric study (m2).

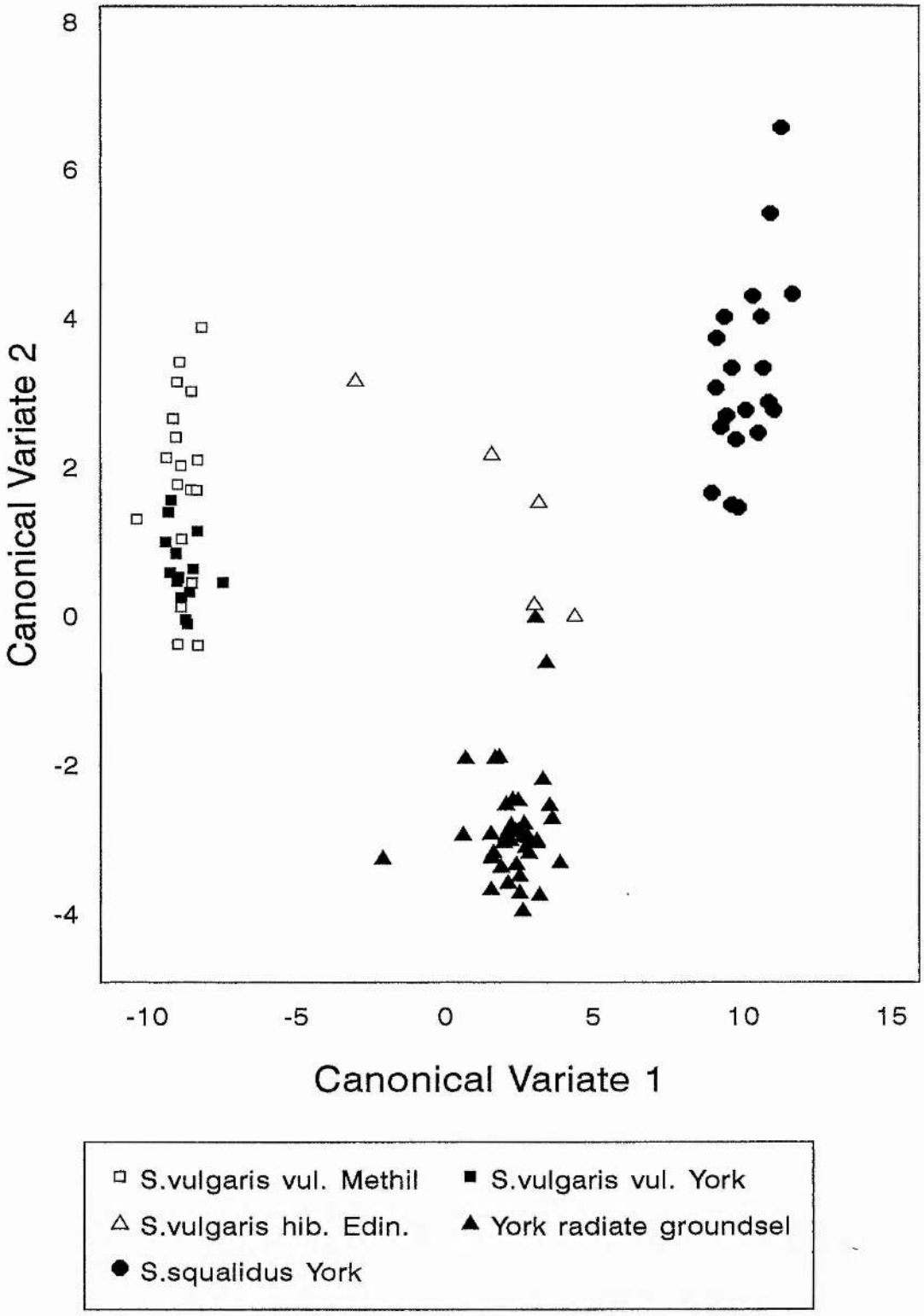


Figure 2.11. A plot of the scores (CV1 vs CV2) for each individual within a given taxon/population following canonical variate analysis of 16 capitulum characters; measured in the second morphometric study. The first two canonical variables were statistically significant ($P < 0.001$), and accounted for 86.4% and 10.9% of the total variance in measurements respectively. The following characters had a high weighting on canonical variable one (with total-standardised canonical coefficients shown in parentheses); C2, inflorescence length (1.936) and C10, number of ray florets (5.905). Canonical variable two was most affected by; C1, plant height (1.064) and C6, number of phyllaries (1.183).

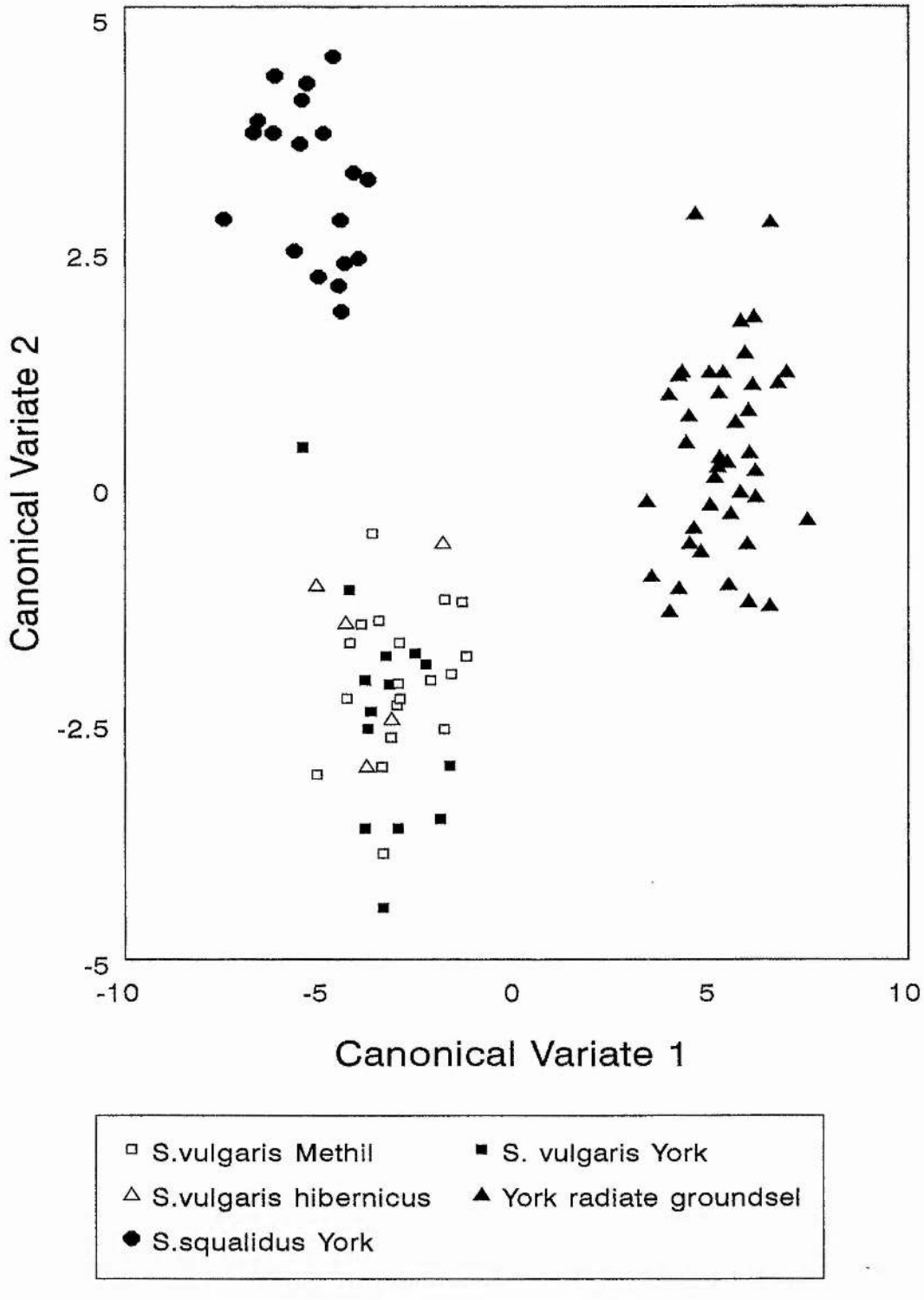


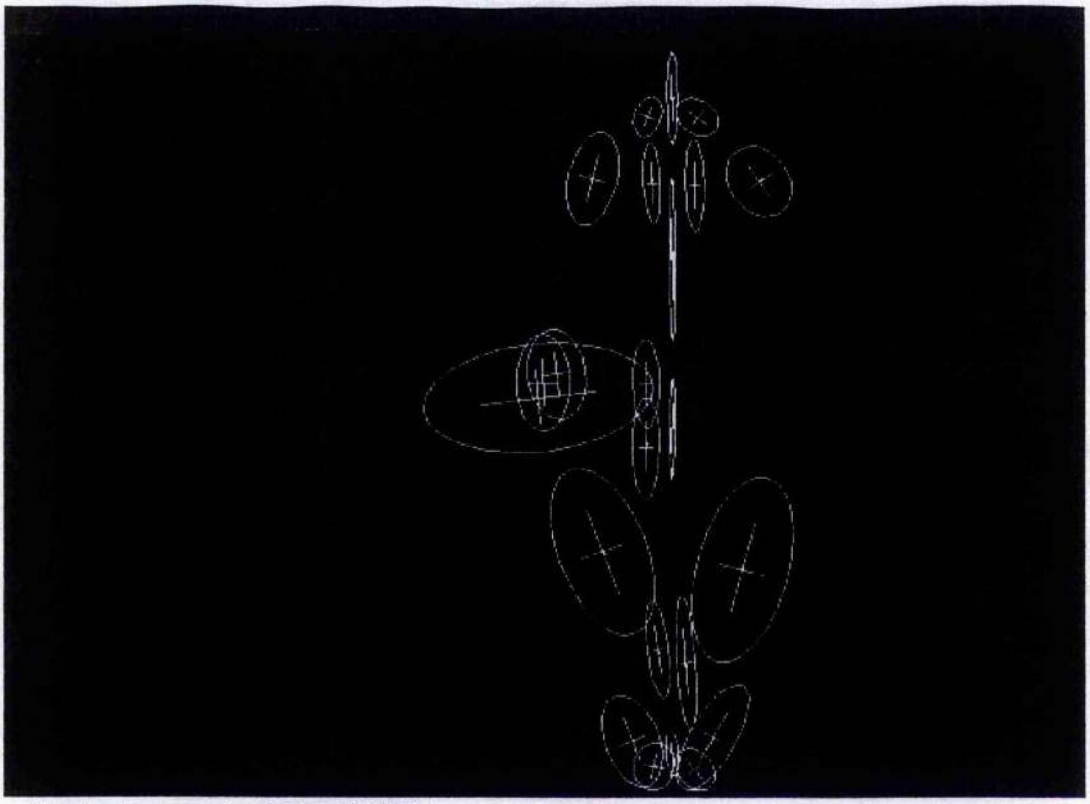
Figure 2.12. A plot of the scores (CV1 vs CV2) for each individual within a given taxon/population following canonical variate analysis of 23 linear and angular midleaf character derived from landmark coordinates; measured in the second morphometric study. The first two canonical variables were statistically significant ($P < 0.001$), and accounted for 80.3.4% and 15.1% of the total variance in measurements respectively. The characters that had a high weighting on canonical variable one (with total-standardised canonical coefficients shown in parentheses) were C15, number of midleaf lobes (1.392); C20, standardized square of leaf area (-1.376); C30, midleaf length (3.388). Canonical variable two was most affected by; C19, standardized leaf perimeter (1.009).

S. vulgaris, placing York radiate plants in an intermediate position. In contrast, plants of *S. vulgaris* var. *hibernicus* remained clustered with plants of var. *vulgaris* based on midleaf morphology.

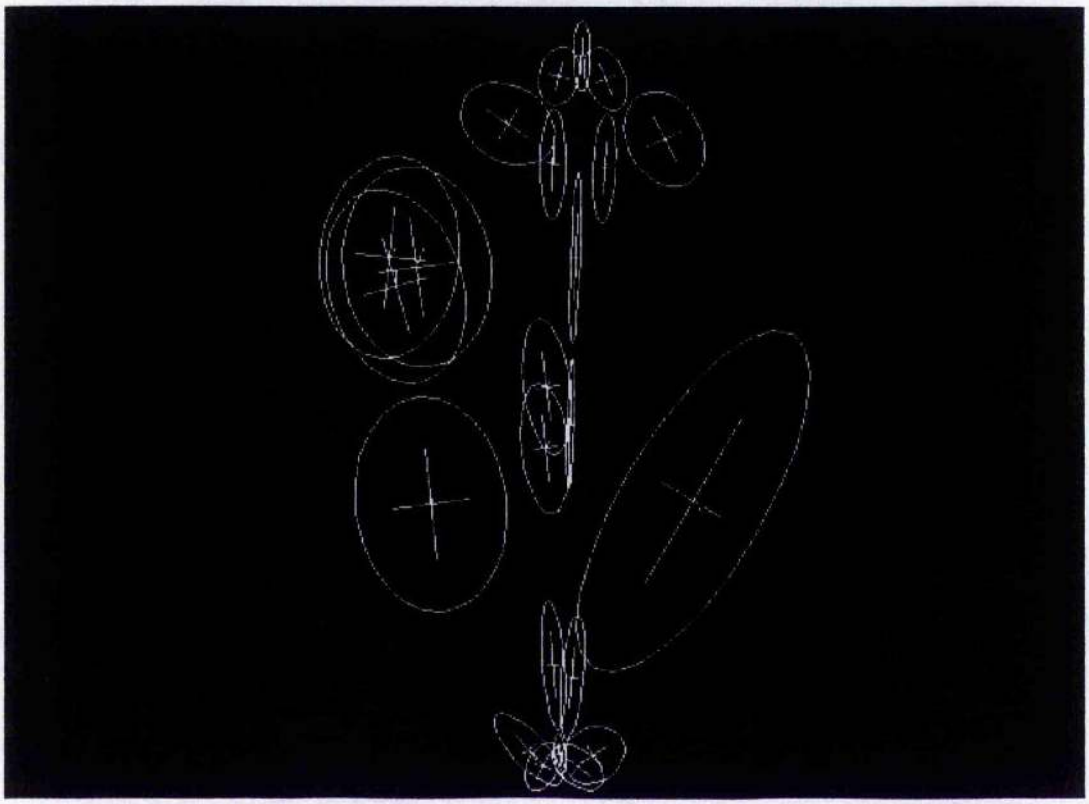
Midleaf landmark analysis

Consensus coordinates for each of the 23 midleaf landmarks are presented with standard deviations for *S. vulgaris* var. *vulgaris* from Methil, *S. squalidus* from York, *S. vulgaris* var. *hibernicus* from Edinburgh and the York radiate groundsel (Figure 2.13). The coordinates of consensus midleaf landmarks for *S. vulgaris* var. *hibernicus* were superimposed onto the coordinates of consensus midleaf landmarks for var. *vulgaris* and the direction of shape change between the two sets of coordinates are represented by vectors in Figures 2.14a. Similarly the vectors describing the relative positional change of York radiate groundsel coordinates following the superimposition of consensus midleaf landmarks onto those of var. *vulgaris* can be seen in Figure 2.14b.

The consensus midleaf landmark coordinates of *S. vulgaris* var. *hibernicus* match very closely to those of var. *vulgaris* with a resistant fit means square of 0.00845 (Table 2.9). However, the consensus midleaf landmark coordinates of York radiate groundsel do not fit as well onto those of *S. vulgaris* var. *vulgaris*, giving a resistant fit means square of 0.01421. York radiate groundsel midleaves exhibit considerable localized elongation of the most-apical landmarks relative to the leaf base and also considerable narrowing of the distance between apical landmarks relative to those of *S. vulgaris* var. *vulgaris* (Figure 2.14b). These observations are consistent with single character analysis by ANOVA where the apical lobe length was significantly longer (C31) and both apical angle A (C42) and the secondary vein angle of the apical adjacent lobe (C44) were significantly more acute for York radiate groundsel than for Methil material of *S. vulgaris* var. *vulgaris*. Both *S. vulgaris* var. *hibernicus* and York radiate groundsel show localized change around the mid-lobe landmarks. However, compared with *S. vulgaris* var. *hibernicus*, York radiate groundsel individuals exhibit greater localized elongation and widening of basal auricle consensus landmark positions relative to the point of stem attachment. This result is again consistent with the findings of the single character analysis which showed York radiate groundsel individuals having a significantly greater basal auricle extension beyond the point of stem attachment (C38) and basal auricle width (C35) relative to var. *vulgaris* individuals.

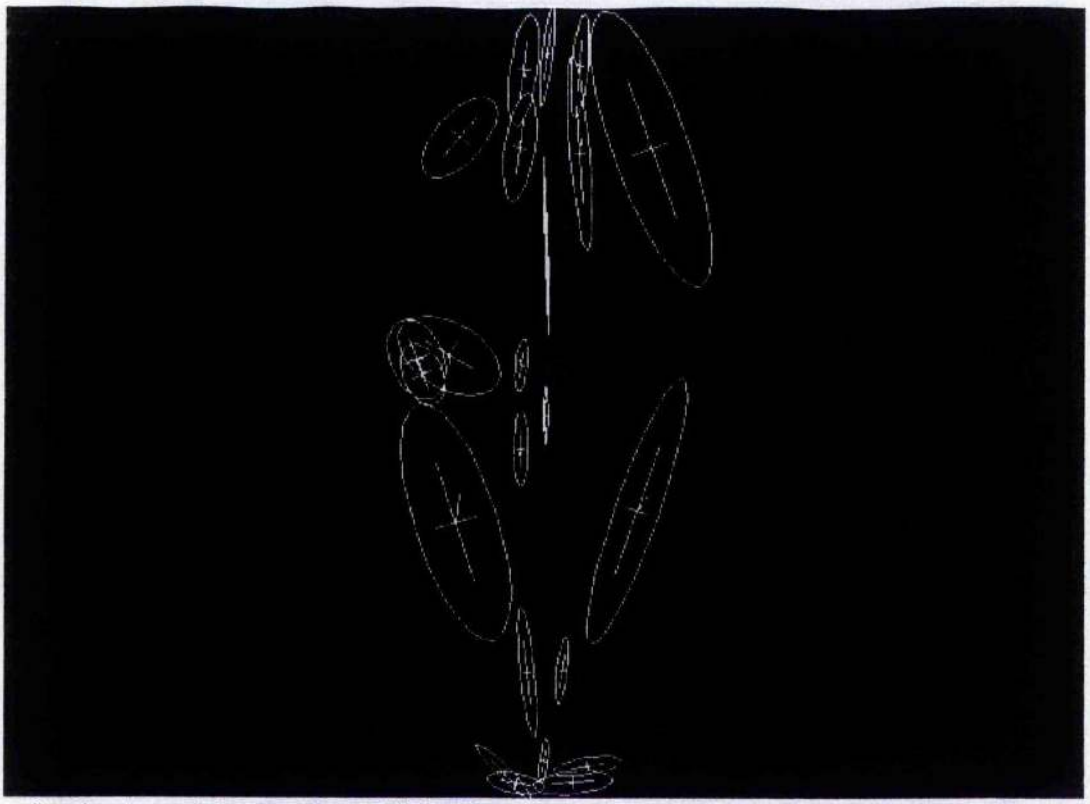


a. *S. vulgaris* var. *vulgaris* from Methil.

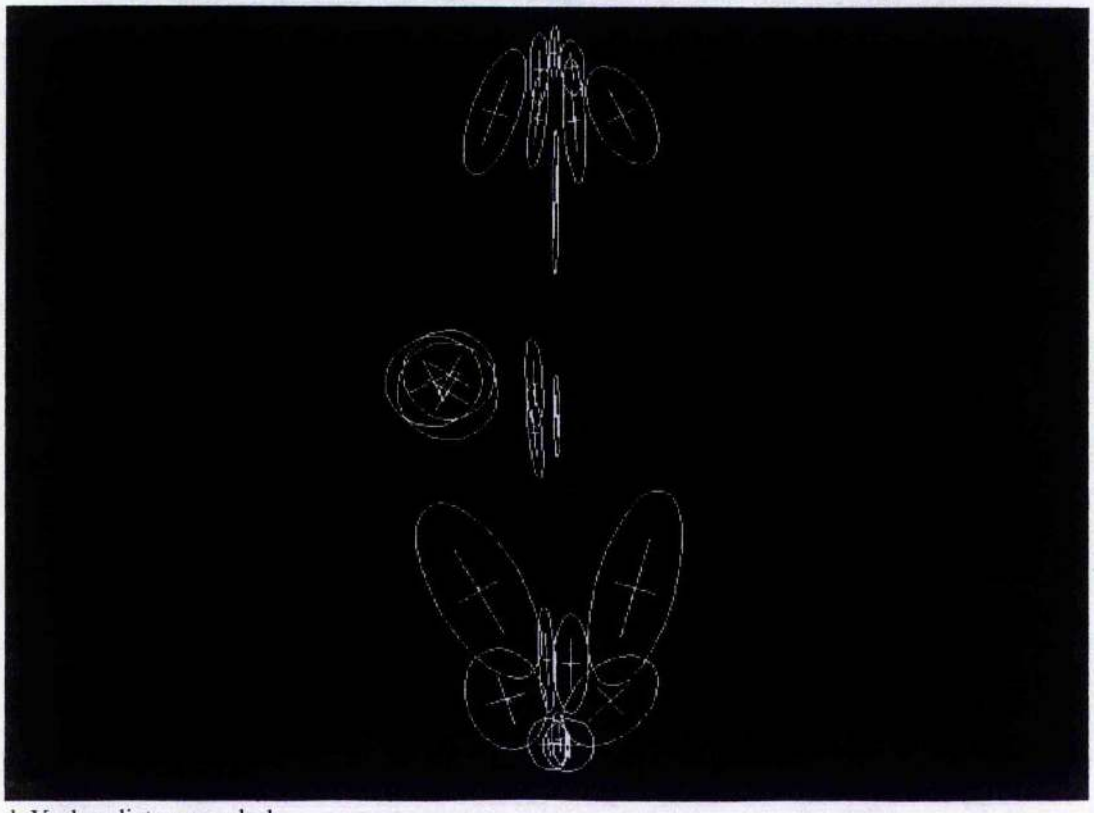


b. *S. squalidus* from York.

Figure 2.13. Plot of consensus coordinates for 23 landmarks measured on midleafs of taxa indicated. Crosses indicate the extent of one standard deviation and the circles two SD units.

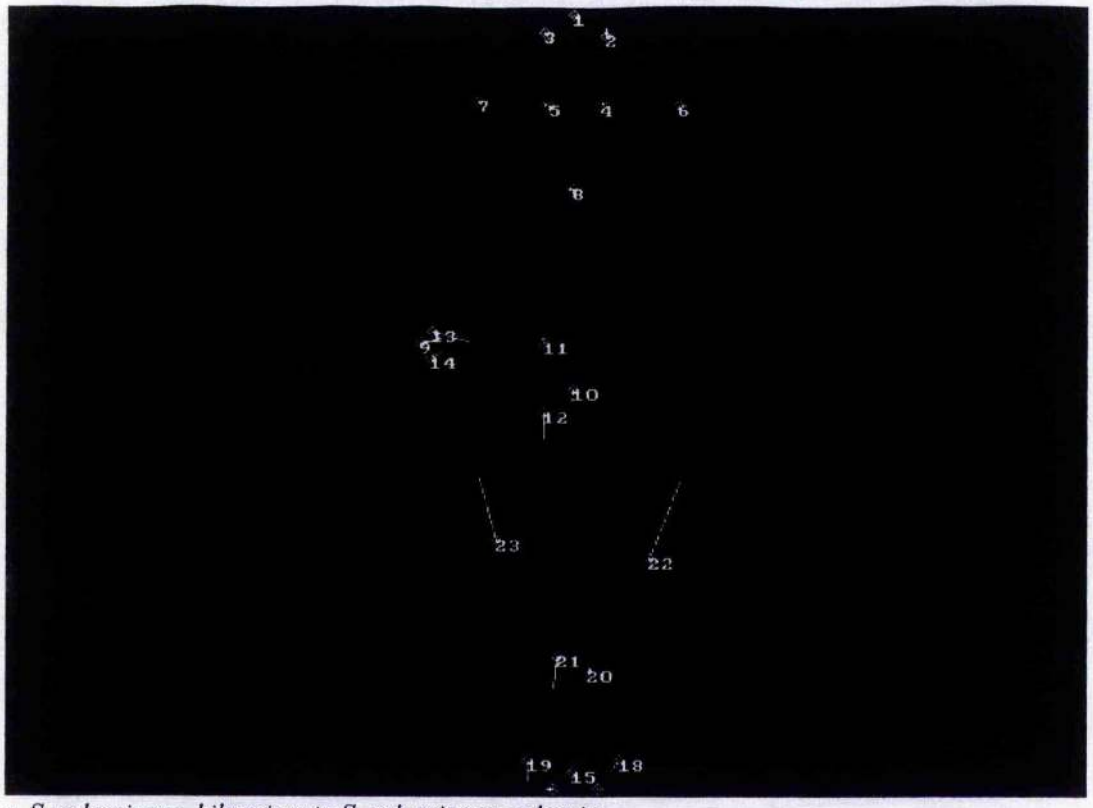


c. *S. vulgaris* var. *hibernicus* from Edinburgh.

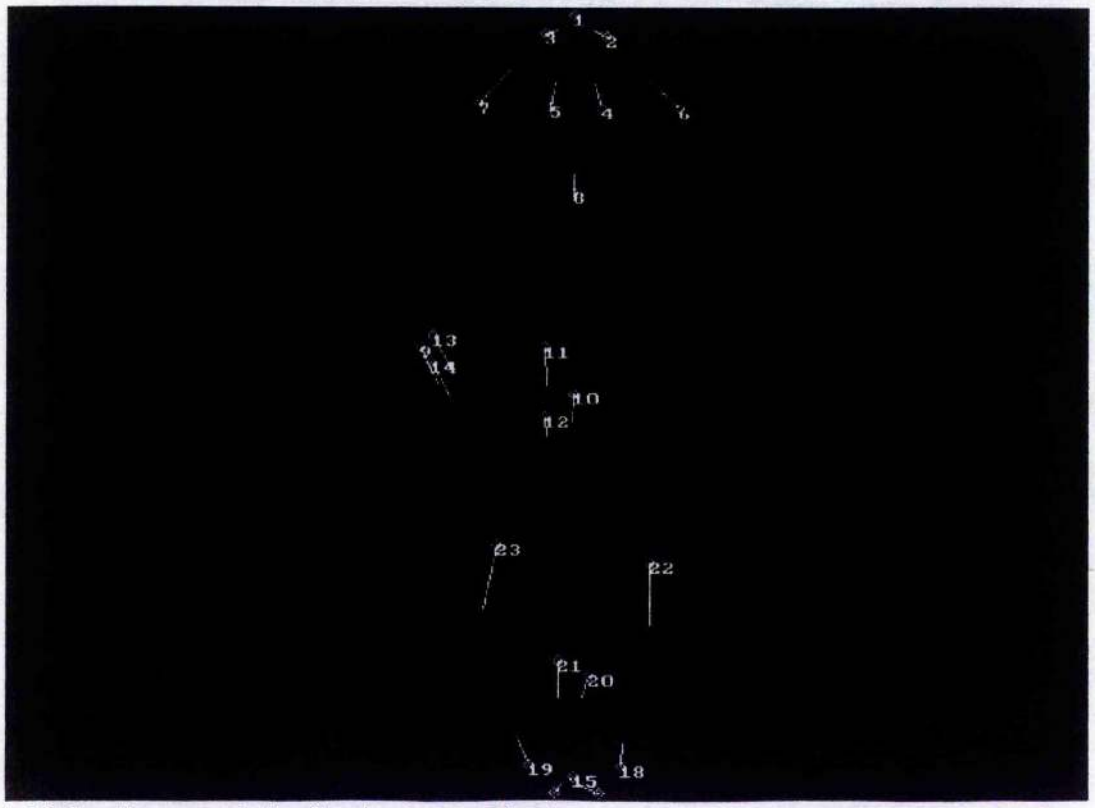


d. York radiate groundsel

Figure 2.13. Continued.



a. *S. vulgaris* var. *hibernicus* to *S. vulgaris* var. *vulgaris*



b. York radiate groundsel to *S. vulgaris* var. *vulgaris*

Figure 2.12. Residual vectors from a generalised resistant fit of the consensus landmark configuration of York radiate groundsel (b) and *S. vulgaris* var. *hibernicus* (a) to the consensus landmark configuration for *S. vulgaris* var. *vulgaris*. The vectors indicate the relative shift of each of the 23 landmark coordinates after fitting.

Table 2.9. Resistant Fit means squares of pairwise superimpositions of the consensus landmark positions on the listed taxa.

Reference taxa Fitted taxa	*S. vulgaris* var. *vulgaris* Methil	*S. squalidus* York
S. squalidus York	0.03396	-
S. vulgaris var. *vulgaris* Methil	-	0.03199
S. vulgaris var. *hibernicus* Leith	0.00845	0.02990
York radiate groundsel	0.01421	0.06549

Chromosome analysis

Unambiguous chromosome counts of clearly visible mitotic root tip cells confirmed that York radiate groundsel was tetraploid with the modal number of chromosomes being 40, see Table 2.10 and Figure 2.15a, b and c. A new finding was the presence of B chromosomes in cells, with most observed cells possessing two B chromosomes per nucleus see Figure 2.15d, e and f. Many cells in early metaphase (including cells for which accurate chromosome counts were not possible) appeared to possess chromosomes distributed into two groups with each group containing approximately the same number of chromosomes (see Figure 2.15 a, b and c).

Isozymes analysis

The isozyme phenotypes resolved in the various taxa examined are illustrated in Figure 2.16. The assignment of phenotypes to particular banding patterns follows Ashton & Abbott (1992a) for Aat-3 and Acp-2, and Abbott, Irwin and Ashton (1992), Ashton & Abbott (1992b) and Irwin & Abbott (1992) for *Gdh-1* and the esterases, α*Est-1*, β*Est-1* and β*Est-3*. In regard to *Aco-1* and *Idh-1*, phenotypic assignment is based on the known substructure of these enzymes (Weeden and Wendel, 1989). Frequencies of the electrophoretic phenotypes in British populations of *S. vulgaris* var. *vulgaris*, var. *hibernicus*, *S. squalidus*, *S. cambrensis* and York radiate groundsel are presented in Table 2.11.

For the eight enzyme systems that could distinguish the parental taxa, all York radiate groundsel individuals normally expressed the α*Est-1a*, β*Est-3c*, *Acp-2a*, *Gdh-1b*, *Idh-1ab*, *Aco-1a*, *Gdh-1b* and *Aat-3ab* phenotypes, which are commonly found in most British populations of *S. vulgaris*, plus the β*Est-1a* phenotype which is diagnostic of *S. squalidus* (see Figure 2.17).

The pattern of phenotypic variation recorded in *S. vulgaris*, *S. squalidus*, *S. cambrensis* and York radiate groundsel populations for *Acp-1*, *Gdh-1*, *Aat-3*, β*Est-1*, β*Est-3* and α*Est-1* agree broadly with previous reports for these taxa by Ashton & Abbott (1992a, 1992b), and Irwin and Abbott (1992). A UPGMA dendrogram constructed from Nei's genetic distances calculated from allozyme phenotype frequencies (Figure 2.18) separated *S. vulgaris* var. *vulgaris* from *S. squalidus*. Populations of *S. vulgaris* var. *hibernicus* clustered with those of var. *vulgaris* while York radiate groundsel populations were placed in a separate cluster with Welsh populations of *S. cambrensis*. This latter grouping was more similar to *S. vulgaris* than *S. squalidus*. Rather surprisingly, the other population of *S. cambrensis* from Edinburgh exhibited greater genetic similarity to *S. squalidus*.

Table 2.10. Frequency of chromosome counts in mitotic cells examined from separate plants of York radiate groundsel.

Chromosome number	number of plants counted
36	2
37	
38	3
39	
40	5
41	
42	1

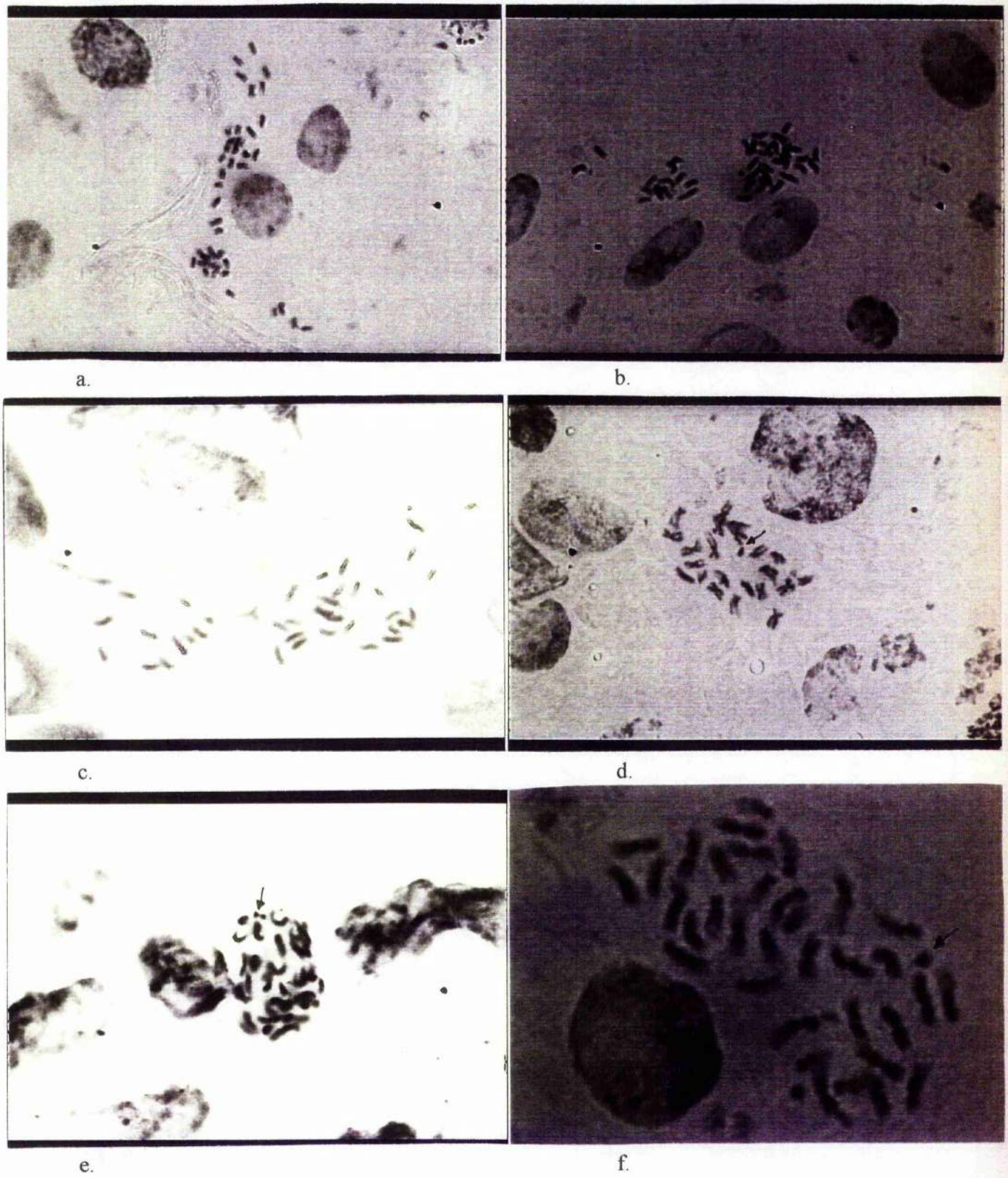


Figure 2.15. Actively dividing root tip cells from York radiate groundsel plants in metaphase I. a, b and c show cells containing 40 chromosomes and two distinct clumpings of chromosomes. d, e and f show evidence of B chromosomes in York radiate groundsel individuals. Cells viewed under oil emersion, magnification x100(f has been enhanced by computer imaging software).

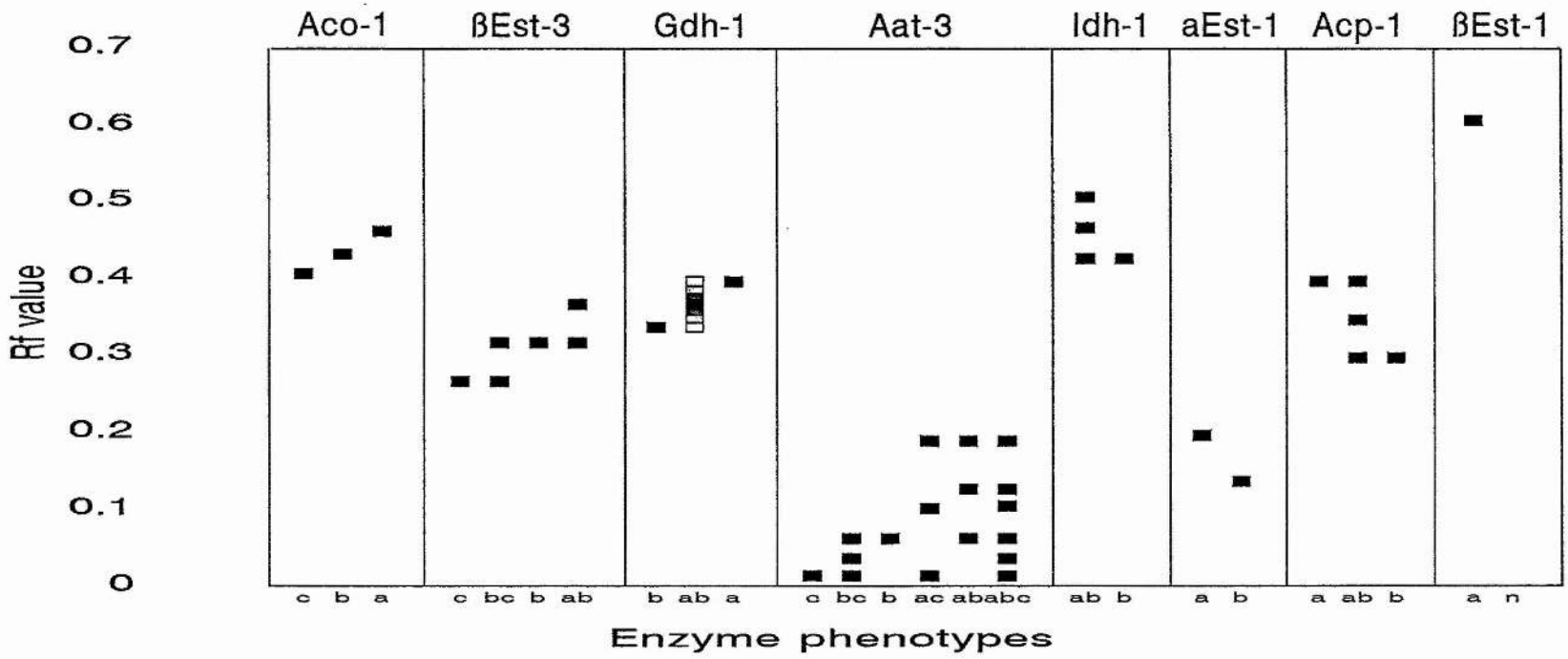


Figure 2.16. Isozyme phenotypes resolved at the enzyme encoding loci; Aco-1, βEst-3, Gdh-1, Aat-3, Idh-1, αEst-1, Acp-1 and βEst-1.

Table 2.11. Allozyme phenotype frequencies in samples of *S. vuglaris* var. *vulgaris*, *S. vulgaris* var. *hibernicus*, *S. squalidus*, *S. cambrensis* and York radiate goundsel collected from the British Isles.

	N=	aEst-1 aa	ab	bb	nn	BEst-3 bb	bc	cc	BEst-1 aa	nn	Acp-2 aa	ab	bb
S.vulgaris var. *vulgaris*													
England													
Bristol	4	1.00	-	-	-	-	1.00	-	-	1.00	1.00	-	-
Dalton Terrace, York	71	1.00	-	-	-	-	-	1.00	0.01	0.99	1.00	-	-
Lendal Bridge, York	38	0.92	0.03	0.05	-	0.23	0.03	0.74	-	1.00	1.00	-	-
Scotland													
Aberfeldy	4	1.00	-	-	-	-	1.00	-	-	1.00	1.00	-	-
Bo'ness	3	0.67	0.33	-	-	-	-	1.00	-	1.00			
Dundee	4	1.00	-	-	-	-	1.00	-	-	1.00	1.00	-	-
Leith, Edinburgh	20	0.85	0.05	0.10	-	-	0.25	0.75	-	1.00	1.00	-	-
Lairg	2	1.00	-	-	-	-	1.00	-	-	1.00	1.00	-	-
Letham Angus	4	1.00	-	-	-	1.00	-	-	-	1.00			
Markinch	4	1.00	-	-	-	-	1.00	-	-	1.00	1.00	-	-
Methil	4	1.00	-	-	-	-	1.00	-	-	1.00			
Wales													
Southsea, Wrexham	4	-	1.00	-	-	-	-	1.00	-	1.00	1.00	-	-
Eire													
Passage West, Cork	9	1.00	-	-	-	-	-	1.00	-	1.00	1.00	-	-
S.vulgaris var. *hibernicus*													
England													
Bristol	3	1.00	-	-	-	-	1.00	-	-	1.00	1.00	-	-
Scotland													
Leith, Edinburgh	19	1.00	-	-	-	-	-	1.00	-	1.00	1.00	-	-
Glasgow	1	1.00	-	-	-	-	-	1.00	-	1.00			
Grangemouth	8	1.00	-	-	-	-	-	1.00	-	1.00			
Wales													
Bangor	2	1.00	-	-	-	-	-	1.00	-	1.00			
Barry	6	1.00	-	-	-	-	-	1.00	-	1.00			
Mochdre	10	1.00	-	-	-	-	-	1.00	-	1.00			
Broughton, Wrexham	6	1.00	-	-	-	-	-	1.00	-	1.00			
Brymbo, Wrexham	1	1.00	-	-	-	-	-	1.00	-	1.00			
Ffrith, Wrexham	3	1.00	-	-	-	-	1.00	-	-	1.00			
Rhostyllen, Wrexham	11	1.00	-	-	-	-	-	1.00	-	1.00			
Southsea, Wrexham	1	1.00	-	-	-	-	-	1.00	-	1.00			
Wrexham	2	1.00	-	-	-	-	-	1.00	-	1.00			
Eire													
Cork	14	1.00	-	-	-	-	-	1.00	-	1.00	1.00	-	-
Passage West, Cork	17	0.23	0.53	0.24	-	-	0.06	0.94	-	1.00	1.00	-	-
York Radiate groundsel													
Dalton Terrace 1993	117	1.00	-	-	-	-	-	1.00	0.97	0.03	1.00	-	-
Lendal Bridge 1991	56	1.00	-	-	-	-	-	1.00	1.00	-	1.00	-	-
Lendal Bridge 1994	23	1.00	-	-	-	-	-	1.00	1.00	-	1.00	-	-
S. cambrensis													
Scotland													
Leith, Edinburgh	17	-	-	1.00	-	-	1.00	-	1.00	-	0.43	0.57	-
Wales													
Mochdre	28	1.00	-	-	-	0.33	0.62	0.05	1.00	-	1.00	-	-
Wrexham	21	1.00	-	-	-	0.43	0.57	-	1.00	-	1.00	-	-
S.squalidus													
England York	14	-	-	-	1.00	-	1.00	-	1.00	-	-	-	1.00
Scotland, Edinburgh	12	-	-	-	1.00	-	1.00	-	1.00	-	-	-	1.00
Eire, Passage West Cork	1	-	-	-	1.00	-	1.00	-	1.00	-	1.00	-	-

Table 2.11 Continued.

	Aat-3						Gdh-1			Aco-1			Idh-1	
	cc	bc	bb	ab	ac	abc	aa	ab	bb	aa	bb	cc	ab	bb
S.vulgaris var. *vulgaris*														
England														
Bristol	-	-	-	1.00	-	-	-	-	1.00	1.00	-	-	1.00	-
Dalton Terrace, York	-	-	-	1.00	-	-	0.80	0.06	0.14	0.99	0.01	-	1.00	-
Lendal Bridge, York	-	-	-	1.00	-	-	0.13	0.08	0.79	0.37	0.60	0.03	1.00	-
Scotland														
Aberfeldy	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
Bo'ness	-	-	-	1.00	-	-	-	-	1.00				1.00	-
Dundee	-	-	-	1.00	-	-	-	-	1.00	1.00	-	-	1.00	-
Leith, Edinburgh	-	0.05	-	0.90	-	0.05	-	-	1.00	-	1.00	-	1.00	-
Lairg	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
Letham Angus	-	-	-	1.00	-	-	-	-	1.00				1.00	-
Markinch	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
Methil	-	-	-	1.00	-	-	-	-	1.00	1.00	-		1.00	-
Wales														
Southsea, Wrexham	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
Eire														
Passage West, Cork	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
S.vulgaris var. *hibernicus*														
England														
Bristol	-	-	-	1.00	-	-	-	-	1.00				1.00	-
Scotland														
Leith, Edinburgh	-	0.42	-	0.42	0.16	-	-	-	1.00	-	1.00	-	1.00	-
Glasgow	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
Grangemouth	-	-	-	0.15	0.85	-	-	-	1.00				1.00	-
Wales														
Bangor	-	-	0.50	0.50	-	-	-	-	1.00				1.00	-
Barry	-	-	-	1.00	-	-	-	-	1.00	-	1.00	-	1.00	-
Mochdre	-	-	-	0.10	0.90	-	-	-	1.00				1.00	-
Broughton, Wrexham	-	1.00	-	-	-	-	-	-	1.00				1.00	-
Brymbo, Wrexham	-	-	-	1.00	-	-	-	-	1.00				1.00	-
Ffrith, Wrexham	-	-	-	-	1.00	-	-	-	1.00				1.00	-
Rhostyllen, Wrexham	0.10	-	-	0.45	0.45	-	-	-	1.00				1.00	-
Southsea, Wrexham	-	-	-	1.00	-	-	-	-	1.00				1.00	-
Wrexham	-	-	-	-	1.00	-	-	-	1.00				1.00	-
Eire														
Cork	-	0.36	-	0.64		-	-	-	1.00	0.36	-	0.64	1.00	-
Passage West, Cork	-	0.12	-	0.88	-	-	1.00	-	-	0.88	0.12	-	1.00	-
York radiate groundsel														
Dalton Terrace 1993	-	-	-	1.00	-	-	0.06	0.02	0.92	0.98	0.02	-	1.00	-
Lendal Bridge 1991	-	-	0.09	0.91	-	-	-	-	1.00	0.96	0.04	-	1.00	-
Lendal Bridge 1994	-	-	-	1.00	-	-	-	-	1.00	0.88	0.12	-	1.00	-
S. cambrensis														
Scotland														
Leith, Edinburgh	-	-	-	-	-	1.00	-	0.35	0.65	-	-	1.00	1.00	-
Wales														
Mochdre	-	-	-	1.00	-	-	-	0.22	0.78	1.00	-	-	1.00	-
Wrexham	-	-	-	-	1.00	-	-	0.19	0.81	1.00	-	-	1.00	-
S.squalidus														
England York	0.14	0.43	0.43	-	-	-	1.00	-	-	-	-	1.00	-	1.00
Scotland Leith, Edinburgh	0.33	0.50	0.17	-	-	-	1.00	-	-	-	-	1.00	-	1.00
Eire Passage West, Cork	-	-	1.00	-	-	-	1.00	-	-	-	-	1.00	-	1.00

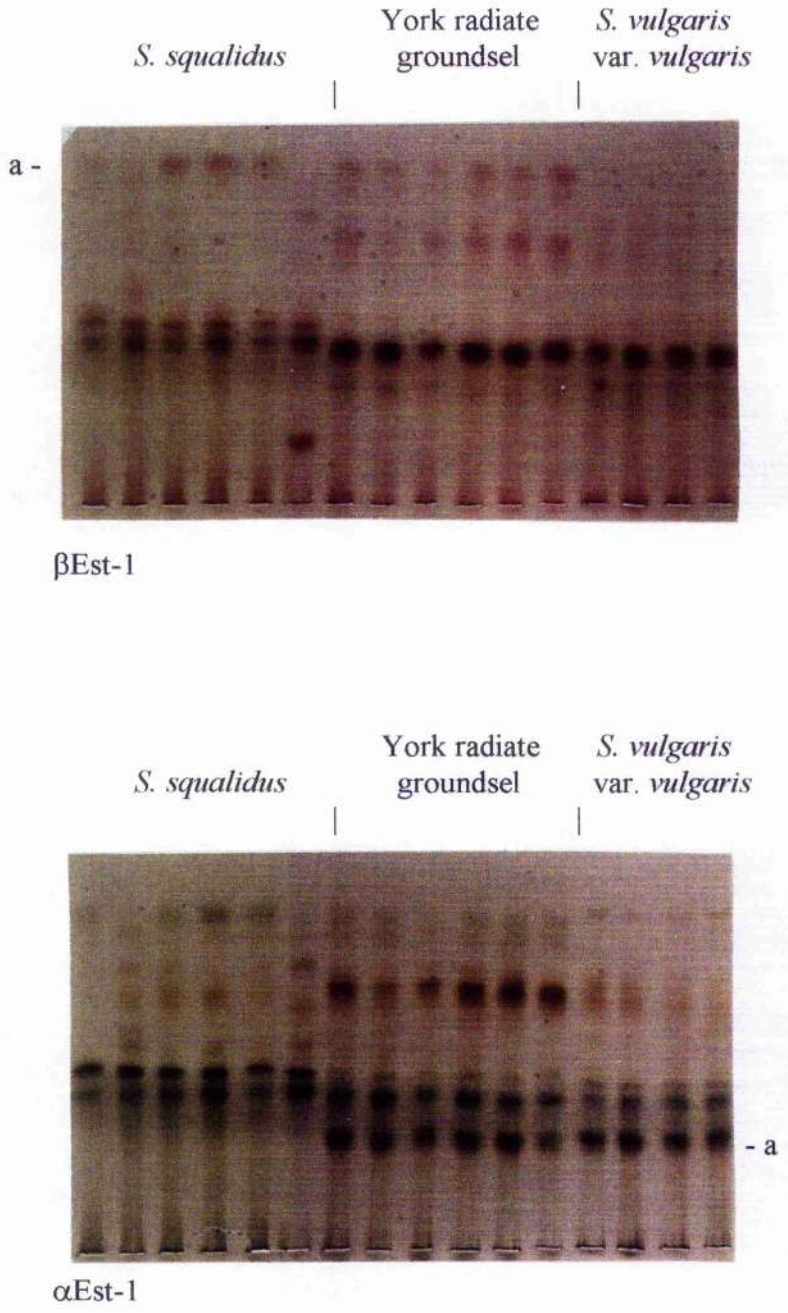


Figure 2.17. Isozyme phenotypes at the βEst-1 and αEst-1 loci for York radiate groundsel, *S. vulgaris* and *S. squalidus*.

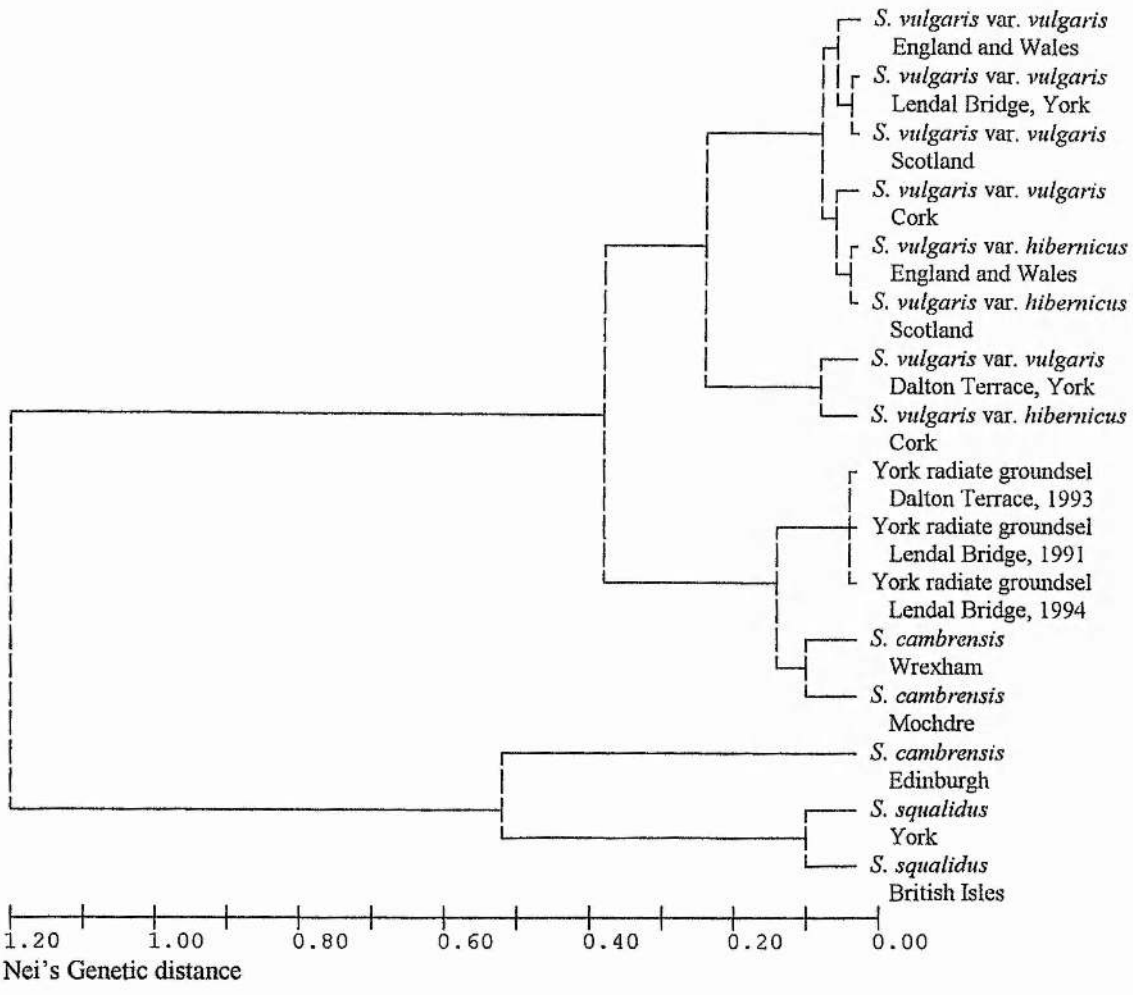


Figure 2.18. Dendrogram produced by UPGMA of Nei's genetic distance (1972) calculated from allozyme phenotype frequency data presented in Table 2.9. Note that allozyme phenotype frequencies were recalculated for *S. vulgaris* var. *vulgaris* (excluding York material) and var. *hibernicus* in England and Wales, and in Scotland after pooling data over samples.

cpDNA RFLP analysis

Restriction analysis of cpDNA revealed that six plants of York radiate groundsel possessed type 3 cpDNA while one had type 2 (Table 2.12). Type 3 cpDNA was also present in two individuals of *S. vulgaris* var. *hibernicus* from Glasgow, while type 2 cpDNA was possessed by both *S. squalidus* individuals surveyed from York in this study and all 22 samples of *S. squalidus* from populations around the British Isles surveyed by Abbott, Curnow and Irwin (1995). Type 2 cpDNA was also found in 12 individuals of *S. vulgaris* var. *vulgaris* and 11 individuals of var. *hibernicus*. The remaining 22 individuals of *S. vulgaris* var. *vulgaris* examined plus four additional individuals of var. *hibernicus*, all possessed type 1 cpDNA.

Ten individuals were chosen from the main analysis to examine six further site and length mutations found to differentiate European *Senecio* taxa by Abbott, Curnow and Irwin (1995) and D. Curnow (unpublished). The distribution of the six additional mutations among taxa can be seen in Table 2.13. The results confirm that for these 10 individuals, the cpDNA haplotypes 1, 2 and 3 correspond exactly to cpDNA haplotypes A, B and C identified by Abbott, Curnow and Irwin (1995).

rDNA RFLP analysis

Restriction analysis of rDNA revealed that the most common length variant in *S. vulgaris* was the *c* variant, which occurred as the only repeat length variant in most accessions of var. *vulgaris* and var. *hibernicus* surveyed (Table 2.14). However, in one individual of var. *vulgaris,* five individuals of var. *hibernicus* and six individuals of York radiate groundsel, the *c* rDNA length variant was present with the *b* length variant (11.65 Kb). The *b* rDNA length variant was found exclusively in five accessions of var. *vulgaris*, one accession of var. *hibernicus* and one accession of York radiate groundsel. The *a* rDNA length variant was unique to *S. squalidus* and found in all but two of the 32 accessions surveyed, although it was usually present in combination with either the *b* or *c* length variants (N.B. *S. squalidus* sample included two York individuals surveyed in this study and 30 individuals from other British populations surveyed by D. Curnow, unpublished).

The distribution of the three restriction site polymorphisms resolved by rDNA restriction analysis is presented in Table 2.14 (also refer to Figure 2.7). All samples of *S. vulgaris* and York radiate groundsel possessed an *Eco*RV restriction site that was absent from individuals of *S. squalidus* (Figure 2.14a). In addition, all individuals of *S. vulgaris* and York radiate groundsel lacked an *Eco*RI restriction site, for which all *S. squalidus* individuals exhibited an additive restriction profile, showing that some

Table 2.12 Summary of the distribution of chloroplast DNA haplotypes 1, 2 and 3 among populations of *S. squalidus*, *S. vulgaris* var. *vulgaris* and var. *hibernicus* and the York radiate groundsel. *Results of 22 *S. squalidus* individuals are from a previous study conducted by Abbott, Curnow and Irwin (1995) and Curnow (unpublished).

	cpDNA type		
Species	1	2	3
S. vulgaris var. *vulgaris* UK	22	12	-
S. vulgaris var. *hibernicus* UK	4	11	2
York radiate groundsel		1	6
S. squalidus UK	-	24*	-

N.B. Locations: var. *vulgaris*: haplotype 1- Aberfeldy, Bo'ness, Bristol, Cork (2), Edinburgh, France, Glasgow, Kingussie, Kirriemuir, Markinch, Perth, Stranraer, Ullapool, Wrexham (4), York (4); haplotype 2- Barry, Dundee, Fort William, Lairg (2), Letham Angus, Methil, York (5).

var. *hibernicus*: haplotype 1- Bristol, Wrexham (3); haplotype 2- Cardiff, Cork (5), Edinburgh, Mochdre, Wrexham (3); haplotype 3- Glasgow (2).

Table 2.13. Chloroplast DNA (cpDNA) haplotype exhibited by ten individuals examined from the main cpDNA analysis for all eight mutations resolved by Abbott, Curnow and Irwin (1995) and Curnow (unpublished) to differentiate European *Senecio* species into cpDNA haplotypes A, B, C, D and E. Numbers of taxon examined are shown in parentheses and results from previous cpDNA surveys are included for comparison (see superscripts).

Mutation type (After Curnow) Restriction enzyme cpDNA probe mutation description Fragment size(s) (kb) present in + Fragment size(s) (kb) present in - Taxa	1 PvuII x pLsC4&5 (site) 9.1 + 4.9 14.0	2 HaeIII x pLsC4&5 (length) .74 .80	3 ClaI x pLsC6 (length) 4.33 4.0	4 ClaI x pLsC6 (site) 3.3 3.1 + (.2)	5 PstI x pLsC1-3 (site) 3.3 3.1 +(.2)	6 CfoI x pLsC6 (site) 8.4 7.0 + 1.4	7 BglII x pLsC7 (site) 3.3 2.9 + (.4)	8 EcoRI pLsC8-11 (site) 2.4 2.0 + (.4)	Haplotype
S.vulgaris var *vulgaris* York (3)	-	-	-	+(2) -(1)	-	?	-	-	B(2) & A(1)
S.vulgaris var. *hibernicus* Glasgow (1)	+	-	+	-	+	?	-	+	C
York radiate groundsel (5)	+	-	+	-	+	?	-	+	C
S.squalidus Britain (1)	-	-	-	+	-	-	-	-	B
S.squalidus Britain (22)[a]	-	-	-	+	-	-	-	-	B
S.vulgaris var. *hibernicus* Edinburgh (1)[a]	-	-	-	-	-	-	-	-	A
S.cambrensis Edinburgh (2)[bc]	-	-	-	-	-	-(1)	-	-	A
S.cambrensis Wales (3)[bc]	+	-	+	-	+	-(1)	-	+	C
S.teneriffae (2)[bc]	+	-	+	-	+	-(1)	-	+	C
S.squalidus Balkans (3)[a]	+	-	+	-	+	-	-	+	C
S.vulgaris Tenerife (1)[c]	+	-	+	-	+	?	-	+	C
S.glaucus spp *cronopofolius* (1)[c]	+	-	+	-	+	?	-	+	C
S.vernalis German (2)[a]	+	-	+	-	+	-	+	+	E
S.vernalis S. European (2)[a]	+	-	+	-	+	-	-	+	C
S.viscosis (2)[a]	-	+	+	-	-	+	-	-	D

[a] All results from Curnow (unpublished)

[b] Including one individual from Curnow (unpublished)

[c] From Lowe and Abbott (1996) and Lowe (unpublished)

Table 2.14. Distribution of ribosomal DNA length variants and site mutations within and amongst samples of *S. vulgaris* var. *vulgaris* and var. *hibernicus*, *S. squalidus* and the York radiate groundsel. Values in parentheses denote the number of individuals of a taxon sharing the rDNA length or restriction site variant.

	Length variants (a-12.4 Kb; b-11.65Kb; c-11.3Kb)	Site mutations and expected fragment sizes EcoRV (+ 4.0 & 0.4 Kb - 4.4 Kb)	EcoRI (+ 3.1 & 0.55 Kb - 3.65 Kb)	BclI (+ 8.4 Kb - length var.)
S. vulgaris var. *vulgaris* British Isles. (33)	b (5), c (27), b/c (1)	+	-	+
S. vulgaris var. *hibernicus* British Isles. (16)	b (1), c (9), b/c (6)	+	-	+
York radiate groundsel (7) York, UK	b(1), b/c(6)	+	-	+(6) +/-(1)
S. squalidus British Isles. (32)[d]	a (1), b (2), a/b (13), a/c (1) a/(b or c) (14), a/b/c (1)	-	+/-	-

[d] Results include those from Curnow, D. (University of St Andrews, unpublished).

N.B. Locations: var. *vulgaris*: repeat length variant *b*- Kingussie, Wrexham, York (2), and Brittany; *b/c*- Edinburgh; *c*- Aberfeldy, Barry, Bo'ness, Bristol, Cork, Dundee, Fort William, Glasgow, Kirriemuir, Lairg (2), Letham Angus, Markinch, Methil, Perth, Stranraer, Ullapool, Wrexham (3), York (7).

var. *hibernicus*: repeat length variant *b*- Cork; *b/c*- Cardiff, Glasgow (2), Mochdre, Wrexham (2); *c*- Bristol, Cork (3), Edinburgh, Wrexham (4).

S. squalidus: repeat length variant *a*- Kirkcaldy[d]; *b*- Hull[d], York; *a/b*- Ainsdale[d], Barry[d], Cardiff[d], Chesil Beach[d], Cork[d], Dartford[d], Derby[d], Exeter[d], Kirriemuir[d], Weymouth[d], Wrexham[d] (3); *a/c*- HMS Osprey[d]; *a/b/c*- York; *a* (*b* or *c*)- Bristol[d] (2), Cork[d] (2), Edinburgh[d] (4), Oxford[d] (3), Weymouth[d] (3).

rDNA repeats possessed the site while others lacked it (Figure 2.14b). Finally, all samples of *S. vulgaris* and six samples of York radiate groundsel possessed a *BclI* restriction site that was not present in the rDNA of *S. squalidus* (Figure 2.14c). However, one individual of York radiate groundsel exhibited an additive rDNA profile for this polymorphism.

RAPD evidence

Of 40 RAPD primers that were initially examined, 12 were found to amplify some DNA fragments that could be used to distinguish *S. vulgaris* var. *vulgaris*, *S. squalidus*, and York radiate groundsel. All fragments amplified by these 12 primers were scored for each individual. The distribution of RAPD fragments amongst the taxa surveyed is presented in Table 2.15. Out of a total of 57 RAPD fragments amplified, 33 clearly differentiated *S. vulgaris* from *S. squalidus*. Of these 33 fragments, all York radiate groundsel individuals exhibited five *S. squalidus* specific fragments, and six *S. vulgaris* var. *vulgaris* specific fragments (see Figure 2.19 for an example). For 18 RAPD fragments, York radiate groundsel expression was polymorphic; in 14 cases some York radiate groundsel individuals exhibited a fragment possessed by all or most *S. vulgaris* var. *vulgaris* individuals, while others lacked the fragment as did *S. squalidus* individuals; and in four cases, some York radiate groundsel individuals expressed the fragment exhibited by all or most *S. squalidus* individuals, while others lacked the fragment, as did *S. vulgaris* var. *vulgaris*. Two RAPD fragments were unique to *S. squalidus*, while another two were unique to *S. vulgaris*. York radiate groundsel individuals did not produce any unique fragments. In contrast to the frequent additive RAPD profile found in York radiate groundsel, *S. vulgaris* var. *hibernicus* individuals exhibited a profile that was identical to var. *vulgaris* for all but one of the RAPD fragments.

All individuals of each taxon for which the full 57 RAPD fragment data set was available, were analysed by principal coordinate analysis (PCO). The first five eigenroots (1, 2.9699; 2, 1.55646; 3, 0.55122; 4, 0.39975; 5, 0.37314) were larger than the average diagonal value (0.33748), and assumed to be significant (Adams, 1995). The first three eigen roots described 38%, 20% and 7% of the total variance, respectively, and the average similarity ratio between all individuals was 0.647. Three main clusters were formed comprising York radiate groundsel, *S. vulgaris* and *S. squalidus* with minimum values of similarity between members within these groups being 0.7719, 0.859 and 0.877, respectively. The group of York radiate groundsel plants was more similar to that of *S. vulgaris* plants than to *S. squalidus*. These two groups clustered at a minimum similarity value greater than 0.684, whereas *S.*

Table 2.15. Presence (+) or absence (-) of 57 RAPD fragments scored from nine RAPD primers for individuals of *S. vulgaris* var. *vulgaris*, var. *hibernicus*, York radiate groundsel and *S. squalidus*. Each RAPD fragment is labelled according to the primer used and the size of the fragment generated, so the first fragment was amplified by primer OPH04 and was appoximately 1130 bp in size. Numbers in parentheses indicate numbers of individuals possessing character state if not fixed in the sample.

RAPD fragment	*S. vulgaris* var. *vulgaris* Methil	*S. vulgaris* var. *vulgaris* York	*S. vulgaris* var. *hibernicus* Edinburgh	York radiate groundsel	*S. squalidus* York
	N=8	4	3	8	1
OPA04-1130	+(6) -(2)	+	+	-	-
OPA04-1050	+(6) -(2)	+	+	-	-
OPA04-900	+(7) -(1)	+	+	+(7) -(1)	+
OPA04-650	-	+	+	+	+(3) -(1)
OPA04-620	+	+	+	+	+(3) -(1)
	N=6	2	4	10	4
OPA09-820	+	+	+	+	+(3) -(1)
OPA09-720	-	-	-	+(7) -(3)	+(2) -(2)
OPA09-550	+	+	+	+	+
OPA09-510	+	+	+	+(1) -(9)	+
OPA09-470	+(3) -(3)	-	-	+(8) -(2)	+
OPA09-350	+(5) -(1)	+	+(1) -(3)	+(8) -(2)	+
	N=6	5	4	10	5
OPA12-910	+(4) -(2)	+(2) -(3)	+	+(6) -(4)	-
OPA12-880	-	+(1) -(4)	+(2) -(2)	+	+(3) -(2)
OPA12-850	+	+	+	-	+(3) -(2)
OPA12-750	+(5) -(1)	+(4) -(1)	+(2) -(3)	+(1) -(9)	+(4) -(1)
OPA12-710	+(1) -(5)	+(1) -(4)	-(2) +(2)	+(6) -(4)	+(4) -(1)
OPA12-550	-	-	-	+(8) -(2)	+(3) -(2)
	N=5	1	2	8	4
OPA15-1000	-	-	-	-	+(2) -(2)
OPA15-850	-	-	-	-	+(2) -(2)
OPA15-700	+	+	+	+(5) -(3)	-
OPA15-450	-	-	-	+	+
OPA15-350	+	+	+	+(1) -(7)	-
	N=6	2	3	9	3
OPA18-850	+	+	+	+(4) -(5)	-
OPA18-750	+(3) -(3)	+(1) -(1)	-	+	+(1) -(2)
OPA18-490	+	+	+	+	+
OPA18-400	+	+	+	+	+
OPA18-320	+	+	+	+	-
OPA18-250	-	-	+(1) -(2)	+	+
	N=6	2	5	10	3
OPA19-650	+	+	+	+	+
OPA19-600	+	+	+	+(2) -(8)	-
OPA19-490	+	+	+	+	-
OPA19-350	+(3) -(3)	+(1) -(1)	+	+	+

Table 2.15. Continued.

RAPD fragment	*S. vulgaris* var. *vulgaris* Methil	*S. vulgaris* var. *vulgaris* York	*S. vulgaris* var. *hibernicus* Edinburgh	York radiate groundsel	*S.squalidus* York
	N=8	5	5	11	5
OPH01-750	+	+(4) -(1)	+(4) -(1)	+(6) -(5)	+(1) -(4)
OPH01-650	+	+	+	+	+(4) -(1)
OPH01-620	-	-	-	+	+(3) -(2)
OPH01-550	-	-	-	+	+
OPH01-520	+	+	+	+	+(4) -(1)
OPH01-450	+	+(4) -(1)	+(4) -(1)	+(6) -(5)	+(1) -(4)
OPH01-400	+(3) -(5)	+	+(2) -(3)	+	+
	N=6	1	4	10	3
OPH02-600	+	+	+(3) -(1)	+(7) -(3)	+
OPH02-500	+(4) -(2)	+	+(3) -(1)	+(5) -(5)	+(2) -(1)
OPH02-420	+	+	+	+(9) -(1)	-
	N=6	1	3	10	3
OPH04-620	+	-	+(2) -(1)	+(5) -(5)	-
OPH04-420	+	+	+	+(7) -(3)	-
OPH04-360	+	+	+	+	+
OPH04-250	+(2) -(4)	-	+(2) -(1)	+(5) -(5)	-
	N=6	2	4	10	4
OPH07-560	+	+(1) -(1)	+	+(1) -(9)	+
OPH07-520	+(5) -(1)	-	+(2) -(2)	+(3) -(7)	-
OPH07-480	+	+	+	+	+
OPH07-350	+	+(1) -(1)	+(3) -(1)	+	-
	N=8	5	5	11	5
OPH08-840	+	+	+	+	-
OPH08-750	+	+	+	+	+
OPH08-600	-	-	-	+	+
	N=5		2	6	4
OPH09-600	+		+	+(5) -(1)	-
OPH09-500	+		+	+	-
OPH09-450	+(3) -(2)		+	+(3) -(3)	-
OPH09-400	+		+	+	-

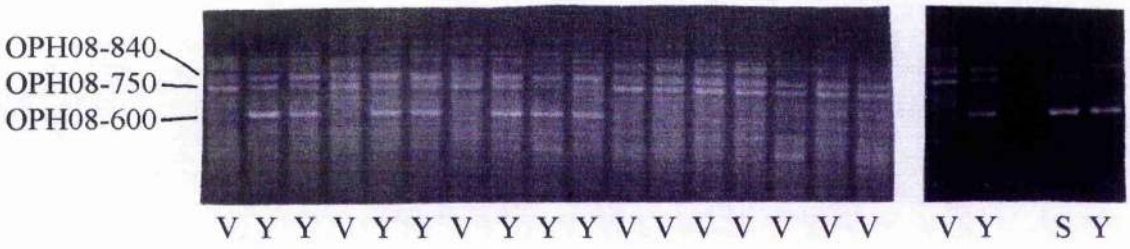


Figure 2.19. Variation in amplification of RAPD fragments observed between *S. vulgaris* and *S. squalidus* using primer OPH08. Fragment OPH08-600 is specific to *S. squalidus* and OPH08-840 to *S. vulgaris*. York radiate groundsel individuals combine the RAPD profile of these parental fragments. Taxa abbreviations; Y, York radiate groundsel; V, *S. vulgaris*; S, *S. squalidus*.

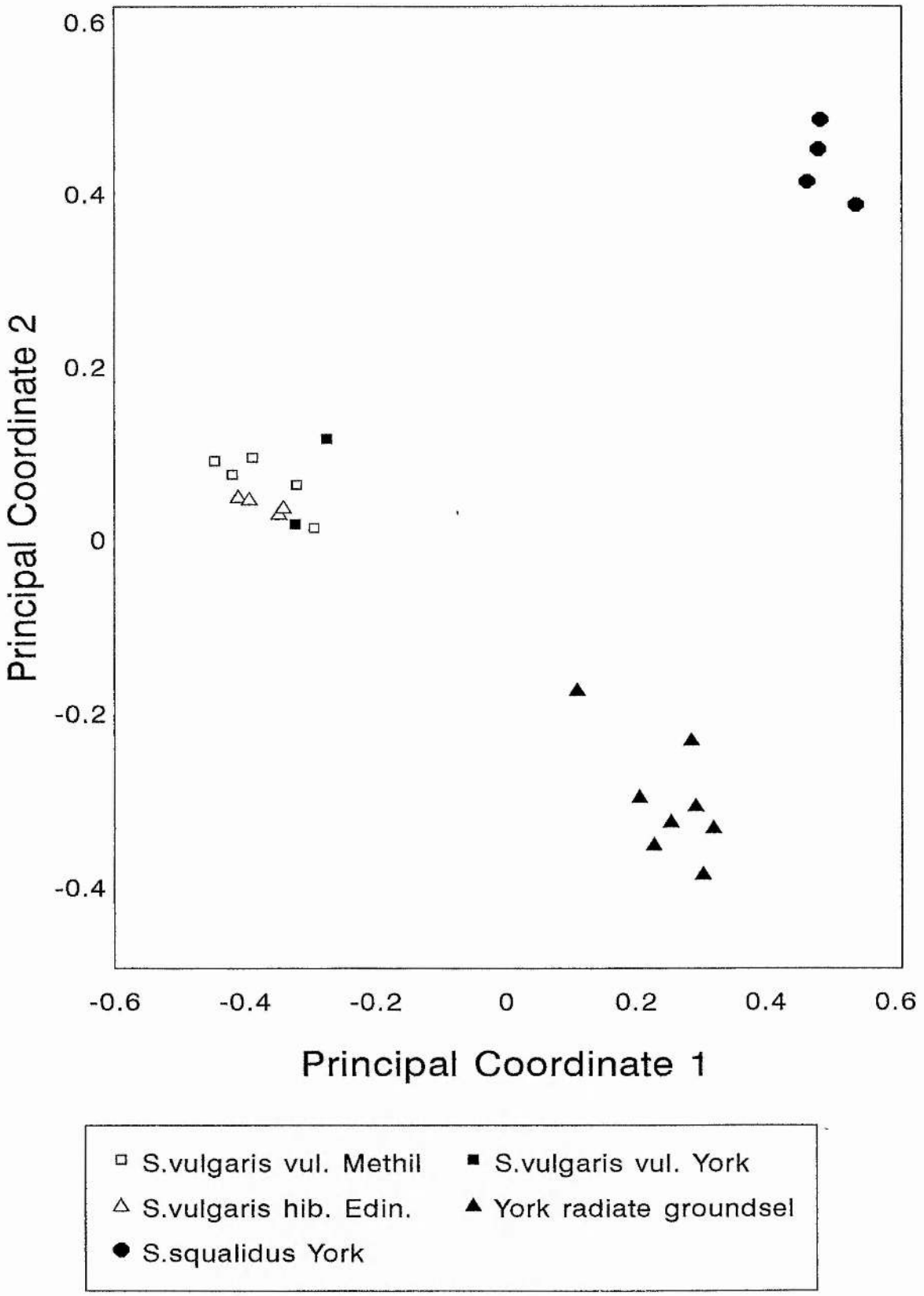


Figure 2.20. A plot of scores (PCO1 vs PCO2) for each individual within a given taxon/population following principal coordinate analysis of similarity measures calculated from the presence and absence of 57 RAPD fragments.

squalidus clustered with the York radiate groundsel and *S. vulgaris* group at a minimum similarity level of 0.632. The first principal coordinate axis (Figure 2.20) separated *S. vulgaris* from *S. squalidus* and placed York radiate groundsel individuals in an intermediate position. Principal coordinate axis two also separated the taxa. In contrast, *S. vulgaris* var. *hibernicus* individuals could not be separated from var. *vulgaris* plants along either axis 1 or 2.

Discussion

The work reported in this chapter provides evidence, from a number of sources, that York radiate groundsel is a hybrid of *S. vulgaris* and *S. squalidus* and is quite distinct from the other known tetraploid hybrid derivative of these two species, *S. vulgaris* var. *hibernicus*.

The hybrid origin of York radiate groundsel; morphological, isozyme and molecular evidence

Single character analysis and multivariate (CVA) analysis of 26 morphological characters showed that, in general, York radiate groundsel exhibited an intermediate morphological phenotype relative to its two putative parental taxa, *S. vulgaris* var. *vulgaris* and *S. squalidus*. In contrast, the stabilized introgressant, *S. vulgaris* var. *hibernicus*, differed from var. *vulgaris* only by characters associated with the presence of ray florets. York radiate groundsel was also shown to possess a number of novel characters not seen in either parental taxon, such that plants typically had long, many lobed leaves, long achenes (>2.5 mm), four pored pollen, a low proportion of phyllaries with black tips and long calyculus bracts. The possession of novel characters by first and later generation hybrids is apparently relatively common in the plant kingdom (Rieseberg and Ellstrand, 1993).

Further examination of leaf morphology, using landmark analysis, showed that the large, many lobed leaves typical of York radiate groundsel were elongated at the apex of each lobe relative to the marginal tooth sinus, giving them a distinctive 'pointed' appearance compared to the leaf shape of *S. vulgaris*. York radiate groundsel individuals also had leaves with a much larger basal auricle than was present in either parental taxa. Taken together, single character ANOVA, canonical variate analysis and resistance fitting of consensus landmark positions, were able to give powerful insights into the differences in midleaf shape between the taxa examined.

In regard to isozyme phenotype, the results of the present study tended to confirm those of Irwin and Abbott (1992), showing that York radiate groundsel possessed a partially additive isozyme profile that combined the ß*Est-1a* allozyme phenotype produced by *S. squalidus*, with allozyme phenotypes produced only by *S. vulgaris* for seven other enzyme systems studied. In contrast, *S. vulgaris* var. *hibernicus* exhibited only those allozyme phenotypes produced by var. *vulgaris*. UPGMA analysis confirmed that over all enzyme systems surveyed, York radiate groundsel was more similar to *S. vulgaris* var. *vulgaris* than *S. squalidus* in isozyme phenotype.

RAPD analysis provided more evidence that York radiate groundsel was genetically intermediate to *S. squalidus* and *S. vulgaris*. Most plants examined expressed five fragments that were diagnostic for *S. squalidus*, together with six fragments only found in *S. vulgaris*. For the remaining 18 fragments that distinguished the parental taxa, York radiate groundsel was polymorphic. No fragments were found in York radiate groundsel that were not present in either parental taxon; however, four fragments expressed by most individuals of the two parents were not produced by the York hybrid. In contrast to what was found for York radiate groundsel, principal coordinate analysis of RAPD profiles could not distinguish *S. vulgaris* var. *hibernicus* from var. *vulgaris*.

RFLP analysis of rDNA revealed that all three restriction enzymes employed (*Bcl*I, *Eco*RI and *Eco*RV) produced rDNA fragment profiles that distinguished *S. squalidus* from *S. vulgaris*. It was a surprise therefore, that only one out of seven York radiate groundsel plants examined exhibited an additive rDNA phenotype (for *Bcl*I, but not *Eco*RI and *Eco*RV), while the remaining plants exhibited an rDNA profile typical of *S. vulgaris* for all three enzymes. However, similar findings were reported by Harris and Ingram (1992a), based on a previous survey of rDNA variation among these taxa. They found that all eleven radiate groundsel plants that they surveyed from York possessed the *S. vulgaris* rDNA phenotype rather than an additive phenotype. The fact that rDNA evidence does not strongly support a hybrid origin of York radiate groundsel is of interest in that it may reflect a peculiar property of the rDNA gene. Other studies have shown that some hybrid taxa frequently possess the rDNA of only one of the putative parents, e.g. *S. cambrensis* (Harris and Ingram, 1992a), *Saxifraga osloensis* (Brochmann, Nilsson and Gabrielsen, 1996), in *Claytonia* (Doyle and Doyle, 1988), in *Lotus* (Liston, Rieseberg and Mistretta, 1990) and F_1 hybrids of *Zea mays* x *Z. luxurians* (Zimmer, Jupe and Walbot, 1988). Presence in a hybrid of only one rDNA parental type may be due to repeated backcrossing of the hybrid to a parent (Rieseberg and Brunsfeld, 1992), or the concerted evolution of rDNA sequences (Brown, Wensink and Jordan, 1972; Zimmer *et al.*, 1980). Concerted evolution involves the non-independent evolution of repetitive DNA sequences, resulting in a sequence similarity of repeating units that is greater within a species than among species (Elder and Turner, 1995). This process is thought to be driven by the poorly understood mechanism of 'molecular drive', where heterogeneous rDNA repeats within a species are quickly homogenized through recombination. Although an additive rDNA phenotype is more usual for plant hybrids (Soltis, Doyle and Soltis, 1992), there is increasing evidence suggesting that concerted evolution is not uncommon in repetitive rDNA sequences, and under certain circumstances, this may make this

molecule unsuitable for use in the study of hybrid origins. Elder and Turner (1995) have argued that any natural grouping of a reproductively isolated organism exhibiting limited geneflow can undergo concerted evolution of repetitive DNA arrays. In view of the fact that York radiate groundsel bears more similarity genetically to *S. vulgaris* than *S. squalidus* (from morphometric, isozyme and RAPD analysis), it seems likely that the distribution of genetic markers exhibited by York radiate groundsel is due to backcrossing with *S. vulgaris* and not just segregation of characters in later generation hybrid progeny. Backcrossing to *S. vulgaris* would reduce the number of *S. squalidus* rDNA sequences, but may not eliminate them totally, it is possible that concerted evolution may have assisted in the elimination of *S. squalidus* rDNA repeats from most York radiate groundsel individuals.

Chloroplast DNA variation among the *Senecio* taxa examined by RFLP analysis, identified three cpDNA haplotypes (types 1, 2 and 3), based on presence/absence of a site and length mutation detected by the *Cla*I x pLsC6 enzyme-probe combination. Further analysis of a limited sample of the same taxa with additional enzyme-probe combinations confirmed that types 1, 2 and 3 were equivalent to cpDNA haplotypes A, B and C described by Abbott, Curnow and Irwin (1995). Six out of seven York radiate groundsel plants analysed were shown to possess type C cpDNA and are assumed to have inherited this type of cpDNA from their maternal parent which most is likely to have been *S. vulgaris*. Previous studies have shown that the cpDNA molecule is maternally inherited in *Senecio* (Harris, 1990), and crosses between *S. vulgaris* and *S. squalidus* are usually only successful when *S. vulgaris* acts as the maternal parent (Ingram, Weir and Abbott, 1980). However, type C cpDNA was not present in any sample of *S. vulgaris* var. *vulgaris*, or *S. squalidus* surveyed in the present study, and a previous study of cpDNA variation (Harris and Ingram, 1992a), also failed to find the 330 bp insert that characterizes the C cpDNA haplotype in either of these British taxa. However, two individuals of *S. vulgaris* var. *hibernicus* from a Glasgow population were found to possess the C cpDNA haplotype, which would indicate that this haplotype is also present in British *S. vulgaris* var. *vulgaris*, but at low frequency.

In contrast to what was found for the majority of York radiate groundsel individuals surveyed, one plant was found to possess type B cpDNA. It is feasible that this plant is the product of a cross in which the maternal plant possessed type B rather than type C cpDNA, which would be evidence for at least two independent origins of the York hybrid. An alternative explanation, however, for the presence of B type cpDNA in York radiate groundsel is that it was introgressed from *S. vulgaris* var. *vulgaris*

following backcrossing. Support for the latter comes from the fact that the York radiate groundsel plant in question also exhibited morphological signs of backcrossing and possessed the null βEst-1 and Gdh-1a allozyme phenotypes, both of which are typical of *S. vulgaris* var. *vulgaris* plants. Harris and Ingram (1992a) also found that one York radiate groundsel individual that they examined lacked the 330-bp cpDNA insert, and therefore, could not have possessed the C haplotype. Although the possibility of a multiple origin of York radiate groundsel cannot be ruled out, it is of interest that the two main extant populations of York radiate groundsel (Dalton Terrace and Lendal Bridge) would not appear to stem from separate origins, as plants in both populations have similar morphology, isozyme profiles and, in general, cpDNA haplotypes.

Stability of York radiate groundsel phenotype over time

The morphometric analysis revealed that, not only were the two populations of York radiate groundsel (Lendal Bridge and Dalton Terrace) very similar, but that plants sampled in 1991 were also very similar to those sampled in 1993. Comparison with the morphometric analysis undertaken by Irwin and Abbott (1992), who examined material sampled from a car park near York railway station in 1979, and Warren (1987), who studied material sampled from the banks of the river Ouse in 1985 and 1986, revealed that some morphological traits have been persistent in York radiate groundsel populations since 1979. The distinctive isozyme profile of York radiate groundsel has also been consistent since 1991, when Irwin and Abbott (1992) sampled material, to 1994 when material in this study was collected. These lines of evidence suggest that, although backcrossing may have been a feature in the derivation of what we now recognize as York radiate groundsel, effective breeding barriers may now exist to preserve its phenotypic identity.

Route of origin of York radiate groundsel

Irwin and Abbott (1992) speculated that there were two most likely routes of origin for York radiate groundsel, either via backcrossing of the triploid *S. vulgaris* x *S. squalidus* hybrid to *S. vulgaris* and resumption of tetraploidy, or via the fusion of a reduced gamete of *S. vulgaris* with an unreduced gamete of *S. squalidus*, generating a tetraploid F_1 hybrid. Morphological, isozyme and molecular markers cannot be used to lend support to either of these routes of origin; however, the chromosome analysis may be informative. B chromosomes were found in most cytological preparations of York radiate groundsel and have been previously reported in *S. squalidus*, but not *S. vulgaris* (F. Bretagnolle, personal communication). It is feasible that B chromosomes in York radiate groundsel have been inherited from *S. squalidus*. Two B

chromosomes were usually seen in York radiate groundsel preparations, as was the case in *S. squalidus*. This might suggest a full chromosome complement of *S. squalidus* (i.e. from an unreduced gamete) is present in the York radiate groundsel. Although care should be taken with this interpretation, as the inheritance of B chromosomes is known to be non-Mendelian (Darlington and LaCour, 1962).

It was also observed that two distinct groupings of chromosomes, of roughly equal size, were evident at early metaphase in actively dividing cells of York radiate groundsel. Other studies have shown that, in hybrids, chromosomes derived from different parental taxa, may form separate groups in the nucleus throughout the cell cycle (Leitch, *et al.*, 1991). If it is assumed that the two groups of chromosomes represent two genomes from separate origins, then it is feasible that a reduced *S. vulgaris* gamete contributed one group and an unreduced *S. squalidus* gamete the other. However, other lines of evidence (morphology, isozyme, rDNA) suggest that York radiate groundsel is not an F_1 hybrid between *S. vulgaris* and *S. squalidus*, but a later generation hybrid that has probably undergone backcrossing to *S. vulgaris*, with which it is now genetically more similar. *S. vulgaris* itself is thought to be of allopolyploid origin (Ingram, 1978; Ashton and Abbott, 1992b), and would also be expected therefore to exhibit some genomic differentiation. The two chromosome groups observed in York radiate groundsel could therefore be mainly due to those expected to be present in *S. vulgaris*.

Possibility of the involvement of other *Senecio* taxa in the origin of York radiate groundsel

In this study, *S. vulgaris* and *S. squalidus* were considered to be the most likely parents of York radiate groundsel and work concentrated on evaluating this hypothesis. The results presented here have established that *S. vulgaris* is one of the parents and the main contributor of genetic material to York radiate groundsel. The involvement of *S. squalidus* as the other parent is also strongly supported, although it is possible that another *Senecio* taxon, closely related to *S. squalidus*, may have been involved. Of 19 native and introduced *Senecio* species that are established in the British Isles (Stace, 1991), only *S. sylvaticus*, *S. viscosus* and *S. vernalis* hybridize either naturally or artificially with *S. vulgaris* or *S. squalidus* (Taylor, 1984; Abbott and Lowe, 1996). *S. viscosus* and *S. sylvaticus* have a hairy, viscid leaf indumentum not seen in any groundsel populations (Taylor, 1984) and both species possess a number of isozyme alleles not found in *S. vulgaris*, *S. squalidus* (Ashton, 1990) or York radiate groundsel (Irwin, 1990; Irwin and Abbott, 1992). These two taxa can, therefore, be omitted from further consideration. However *S. vernalis* remains a

remote contender as a taxon involved in the origin of York radiate groundsel. Although only rarely found in the UK, there is evidence that *S. vernalis* is currently extending its range of distribution into the British Isles (Kadereit, 1983). Hybrids between certain German populations of *S. vernalis,* a likely source of occasional introductions, and British *S. vulgaris* could produce an isozyme profile similar to that typically observed in York radiate groundsel individuals (Ashton, 1990; King, 1994).

However, other lines of evidence indicate that *S. vernalis* could not have been involved in the origin of York radiate groundsel. In a morphometric study, Taylor (1984) was able to synthesize tetraploid hybrid progeny between *S. squalidus* and *S. vulgaris* some of which, from Taylor's descriptions, were very similar morphologically to York radiate groundsel, and were placed in an intermediate position between the parental taxa in a PCA analysis. Comes and Kadereit (1990) have synthesized fertile, tetraploid hybrids from crosses between *S. vulgaris* and *S. vernalis* and these were quite dissimilar to York radiate groundsel individuals in morphology (H.P. Comes, personal communication). Other evidence from previous RFLP analysis of rDNA, showed that both *S. vernalis* individuals surveyed from Austria and Germany, possessed the *BclI* site mutation that is also present in *S. vulgaris* (D. Curnow, unpublished), crossing of these two species would not produce the additive rDNA profile exhibited by one individual of York radiate groundsel. Finally, RFLP analysis of cpDNA found that although type C cpDNA is common in southern European populations of *S. vernalis*, populations in Germany and Austria possessed cpDNA type E (Curnow, unpublished), and so could not have been the original maternal parent of York radiate groundsel (although this does not rule out paternal involvement). In addition, no fertile hybrid between *S. vernalis* and *S. vulgaris* has ever been found in populations on the continent where the two species occur sympatrically (Comes and Kadereit, 1990). Consequently, it is very unlikely that *S. vernalis* could have been a parent of York radiate groundsel.

Chapter 3.
Pathway of origin of York radiate groundsel

Introduction

Interspecific hybridization is now widely accepted as a major route in the evolution of plant species, leading to the origin of stabilized introgressants and homoploid and allopolyploid hybrid species. Several recent reviews have highlighted the frequency and importance of this phenomenon (Abbott, 1992; Arnold, 1992; Rieseberg and Wendel, 1993; Soltis and Soltis, 1993; Arnold and Hodges, 1995), and case studies of introgression and the evolution of hybrid species or stabilized introgressants have been well documented in *Helianthus* (Rieseberg, 1991), *Iris* (Arnold, Hamrick and Bennett, 1990), *Senecio* (Abbott and Lowe, 1996), *Spartina* (Raybould *et al.*, 1991) and *Tragopogon* (Soltis and Soltis, 1989).

Hybridization amongst British *Senecio* species

Ellstrand, Whitkus and Rieseberg (1996) recently reported that the occurrence of spontaneous hybrids is non-randomly distributed amongst taxa, and that in the British Isles, the Asteraceae contained disproportionately more hybrid taxa than most other families. Although *Senecio* is not the most hybrid rich genus in the Asteraceae, it contains many examples of interspecific hybridization, several of which have been studied in detail in the British Isles (Crisp, 1972; Benoit, Crisp and Jones, 1975; Ingram, 1977; Ingram, 1978; Ingram, Weir and Abbott, 1980; Ashton and Abbott, 1992a, 1992b; Abbott, Ashton and Forbes, 1992; Harris and Ingram, 1992a; Irwin and Abbott, 1992). Most of these examples are the result of hybridization following the introduction of an alien taxon to the native flora, causing Abbott (1992) to note that the lack of isolating mechanisms may explain the proliferation of hybridization following the introduction of an alien species. Stace (1991) listed eight *Senecio* species native to the British Isles (*S. aquaticus, S. erucifolius, S. jacobaea, S. paludosus, S. sylvaticus, S. viscosus, S. vulgaris, S. cambrensis*) and 11 introduced species (*S. squalidus, S. vernalis, S. cineraria, S. doria, S. fluviatilis, S. inaequidens, S. smithii, S. doronicum, S. grandiflous, S. ovatus* and *S. pterophorus*). Seven interspecific hybrids between these species have been reported from the wild, of which four have been subjected to detailed examination. These latter four hybrids are derived from crosses between *S. squalidus, S. vulgaris, S. viscosus, S. vernalis* and *S. sylvaticus*. They are: *S. sylvaticus* x *S. viscosus* = *S.* x *viscidulus* Scheele, *S. vulgaris* x *S. vernalis* = *S.* x *helwingii* Beger ex Hegi., *S. squalidus* x *S. viscosus* = *S.* x *subnebrodensis* Simonkai, and *S. squalidus* x *S. vulgaris* = *S.* x *baxteri* Druce. The other three *Senecio* hybrids are products of crosses between *S. jacobaea, S. aquaticus,*

S. erosus and *S. cineraria*, these are; *S. cineraria* x *S. jacobaea* = *S. x albescens* Burb. and Colgan, *S. jacobaea* x *S. aquaticus* = *S.* x *ostenfeldii* Druce, and *S. cineraria* x *S. erucifolius* = *S.* x *thuretii* Briq. and Cavill. These particular hybrids have not yet been subjected to detailed examination, and the parent species are not believed to be capable of hybridizing with the parents of the other four hybrids (Benoit, Crisp and Jones, 1975; Stace, 1991).

Frequency of common British Senecio hybrids

The hybrids *S.* x *subnebrodensis* (first described by J.E. Lousley in 1946 as *S.* x *londinensis*) and *S.* x *viscidulus* have been recorded frequently in Britain (Benoit, Crisp and Jones, 1975). However *S.* x *helwingii* has been recorded rarely, most likely due to the rarity of one of its parents, *S. vernalis*. The frequency of this hybrid may increase in the future, as there is evidence that *S. vernalis* is currently extending its range in Britain (Kadereit, 1983). *S. vulgaris* and *S. vernalis* occur frequently in mixed stands elsewhere in Europe and form hybrids regularly. Comes (1994) documented the distribution of *S. vulgaris* and *S. vernalis* in Israel and noted that *S.* x *helwingii* occurred at frequencies of up to 9.8% in mixed populations, as compared to central Europe where the same hybrid was recorded at frequencies of up to 1.5% (Comes and Kadereit, 1990). *Senecio x baxteri*, the triploid hybrid between *S. squalidus* and *S. vulgaris*, has been recorded commonly in the British Isles (Benoit, Crisp and Jones, 1975). A survey of herbarium material and reports in botanical exchange journals by Crisp (1972), yielded 31 *S.* x *baxteri* specimens. P. Crisp based his identifications of the hybrid on intermediate morphological traits and sterility, and his list of hybrids probably represents a reliable estimate of the number of specimens in herbarium collections at the time. However, Stace (1977) pointed out that few *S.* x *baxteri* specimens have been cytotyped and that the hybrid had been over-recorded in the past, mainly due to confusion with radiate forms of *S. vulgaris*. From a personal survey of herbarium specimens from Reading (RNG), Kew (K), Cambridge (CGE), British Museum (BM), Bristol Museum (BRISTM), Leicester (LTR), Liverpool University (LIVU), Irish National Herbarium (DBN), Manchester (MANCH) and Oxford (OXF), and also taking into account specimens identified by Crisp (1972), I have documented records for a total of 66 *S.* x *baxteri* specimens, based on the possession of abortive achenes and an intermediate or hybrid leaf morphology (Table 3.1). Hybrids also tended to be large, robust individuals. Examples of herbarium specimens of *S. vulgaris* var. *vulgaris*, *S. squalidus* and *S.* x *baxteri* are illustrated in Figure 3.1a, b, c respectively.

Table 3.1. Chronological list of putative specimens of *S* x *baxteri* identified from herbaria (source) at Reading (RNG), Kew (K), Cambridge (CGE), British Museum (BM), Britol Museum (BRISTM), Leicester (LTR), Liverpool University (LIVU), Irish National Herbarium (DBN), Manchester (MANCH) and Oxford (OXF). Specimens also include those identified only by Crisp (1972) and references can be found therin. Specimens identified by P. Crisp and seen by me are acknowledged in the comments. *S.* x *baxteri* specimens were identified on the basis of being totally sterile (see achenes) and having a hybrid or intermediate leaf shape (see leaf; h=hybrid).

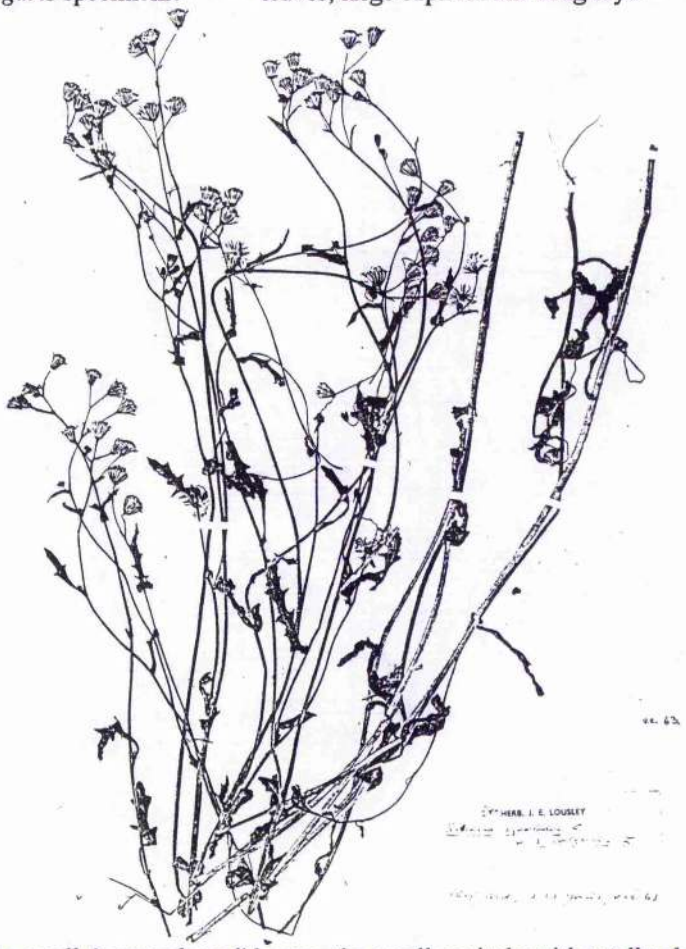
Year	Locality	Source	Achenes	Leaf	Comments
1867	Oxford	BM	sterile	h	'*S.squalidus* var. *parviflorus* Dyer' ref 30341
1884	Jersey	BM	sterile	h	'*S.vulgaris*' ref 30400
1884	Oxford	CGE	sterile		G.C.Druce. det A.C.Leslie
1886	Oxford	BRISTM	sterile	h	G.C.Druce
1886	Oxford	BM	sterile	h	'*S.squalidus x vulgaris, S.crassifolius* Willd' G.C.Druce ref 30317
1886	Oxford	BM	sterile	h	'S.crassifolius *Willd' G.C.Druce ref 30318*
1889	Oxford	K	sterile	h	Type specimen Lond. Bot Exch Club 1898 p9
1891	Oxford	CGE	sterile	h	G.C.Druce
1901	Cork	BM	sterile	h	'*S.squalidus* x *S.vulgaris*' ref 30267 F.W.Burbridge
1905	Cardiff	BM	sterile	h	'*S.vernalis*'H.J.Riddelsdell. ref 30581, 30571-3; 4 specimens. at CGE det. A.C.Leslie & P.D.Sell
1906	Cardiff	BM	sterile	h	'*S.squalidus* x *S.vulgaris*' ref 30599 and 30598; two specimens. at CGE det. A.C.Leslie
1907	Reading	BM	sterile	h	'*S.squalidus* x *S.vulgaris*' ref 30440 Jackson
1910	Cardiff	BM	sterile	h	'*S.squalidus* x *S.vulgaris*' ref 30601
1911	Oxford	CGE	sterile	h	Crisp 1972
1920	Cardiff	BRISTM	sterile	h	E.Armitage
1927	Avon mouth	Crisp 72			det. N.Sandwith *RBEC* (1932):341.
1930	Dorset	K	sterile	h	M.J.Andrews. right specimen only *S. x baxterii*
1933	Southsea	BRISTM	sterile	h	'*S.squalidus*' I.W.Evans
1934	Avonmouth	RNG	?	?	seedlings? H.S.Thompson
1938	Rotherham, Yorks	BM	sterile	h	'*S.vulgaris*' Biggin ref 30231
1941	Goodrington	K	sterile	h	Crisp 1972
1943	London	BM	sterile	h	Crisp 1972
1943	Norwich	RNG	sterile	h	'general appearance indistinguishable from *S.vulgaris* with rayed florets'HJHoward&EAEllis
1944	Bristol	Crisp 72			*RBEC* (1945):60 det. Sandwith
1944	London	K	sterile	h	'8-13 rays 3.5-4x2mm, achenes globescent or shortly sparcely pubescent' N.Y.Sandwith
1944	London	Crisp 72			*RBEC* (1945):60
1944	Norwich	LTR	sterile	h	E.A.Ellis. G76
1945	Stoney Hill	LTR	sterile	h	*S.vulgaris* x *squalidus* with parents-Gibbons and Bell. 'may be form of *S.squalidus*'-NJS
1946	Eastborne	RNG	sterile	h	W.G.L.Sladen
1946	Eastborne	K	sterile	s	'abortive achenes, leaves small shivelled either rotten or *S.subnebrodensis*' WJ.C.Sloden
1946	Eastborne	K	sterile	h	W.J.C.Sloden. hybrid *S. vulgaris* type leaves.
1946	Norwich	RNG	sterile	h	'differs from *radiatus* in; size, part of v. large plant; abortive achenes; leaf shape' E.A.Ellis
1946	Norwich	K, CGE	sterile	h	'differs from *radiatus* in large size, abortive achenes & leaf shape' E.A.Ellis

Table 3.1. Continued.

1946	Norwich	BM	sterile	h	ref 30345
1946	Oxford	RNG	sterile	h	J.P.M.Brenan. see note
1946	Oxford	K	sterile	h	large plant, ligules 4x1.75mm, long stimagtic tuft, achenes sparsely, irregularly pubescent
1946	Oxford	K	sterile	h	J.P.M.Breman
1946	Oxford	BM	sterile	h	*'S.squalidus* x *S.vulgaris'* ref 30277 Chaffle
1947	Gloucester	K	sterile	sq	C.C.Townsend. looks more like *S.squalidus*
1947	Walthampstow	Crisp 72			*Lond. Nat. Suppl.* (1953):32 det. Lousley and D.H.Kent
1948	Bristol	BRISTM	sterile	h	I.W.Evans
1948	Hampstead Heath	RNG	sterile	h	'yellow rays rather short, achenes apparently abortive' J.E.Lousley
1948	Hounslow	K	sterile	h	'large plant, wide branching, achenes glabrous, 10-13rays'B.Welch. 'near to *squal'*NYS
1948	London	Crisp 72			*Lond. Nat. Suppl.* (1953):32 det. Sandwith
1948	Wolverhampton	RNG	sterile	h	'part of very large bushy plant' LNHS
1949	Southhampton	BM	sterile	h	*'S.squalidus* x *S.vulgaris'* ref 30184 A.H.G.Alsten
1949	Tewkesbury	RNG	sterile	h	'hybrid petals short 5-6mm, obtuse, all specimens part of very large plant' E.W.Barriston
1949	Whitley	BM			det. Lousley. P. Crisp
1949	Yardley, Worcs	BM	sterile	h	*'S.squalidus* x *S.vulgaris'* ref 30441 V.Jacobs
1950	Hull	Crackles	sterile	h	det. J.E.Lousley. Crackles 1990 plus two other specimens in 1952.
1951	Sheffield	BM	sterile	h	ref. 30484
1951	Sheffield	BM, CGE	sterile	h	*'S.squalidus* x *S.vulgaris'* ref 30483
1958	Hurworth	Crisp 72			*Vasculum* **43**:7 (1958). det. J.W. Heslop-Harrison
1958	Ness, Ches	LIV	sterile?	h	*'S.cambrensis'* H.E.Green. certainly isn't *S.cambrensis*
1959	Raishy	Crisp 72			*Vasculum* **44**:15 (1958). det. J.W. Heslop-Harrison
1962	Seven oaks	CGE	sterile	h	det. A.C.Leslie
1964	Kew	K	sterile	h	'nice leaves!'
1964	Kew	K	sterile	h	'nice leaves!'
1969	London	Crisp 72			*Lond. Nat.* **41**:21 1961 det. D.E.Allen
1971	Manchester Uni	LTR	sterile	h	C.A.Stace. sterile, leaves int.
1971	Manchester Uni	LTR	sterile	h	'v.rare among abundance of both parents, not seen before this year' C.A.Stace. sterile, int.
1974	Manchester Uni	LTR	sterile	h	'2n=30. Leaves very distinctive-separate this hybrid from all other variants of *vulgaris'* CAS
1974	Norwich	LTR	sterile	h	*'S.squalidus* x *vulgaris* sterile plant F1 cross' E.H.Davids.
1977	Liverpool	LIV	sterile	h	*'S.squalidus* x *vulgaris'* A.J.Coombes four specimens
1951	Sheffield	RNG	sterile	h	'woody, infertile, large ligules-*S.squalidus* x *vulgaris'* John Moun
1977	Toft, Cambs	CGE	sterile	h	'one large bushy plant' root tips 2n=30, confirmed cytologically R.I.SBretnall & A.C.Leslie

a. *S. vulgaris* var. *vulgaris* (RNG), showing typical leaf shape of var. *vulgaris* specimens.

b. *S. squalidus* (KEW) showing highly dissected leaves, large capitula and long rays.



c. *S.* x *baxteri* (BM), note the small, 'arrow shaped' leaves, the small capitula with totally abortive achenes and the presence of small rays. This specimen is part of a large robust plant.

Figure 3.1. Herbarium specimens of indicated taxa

Estimates of the frequency of interspecific hybridization between *S. squalidus* and *S. vulgaris* in large mixed populations have been obtained by Marshall and Abbott (1980). They recorded eight, cytologically confirmed, triploid hybrids among a total of approximately 30,000 individuals of *S. vulgaris* at four waste sites in the British Isles. Furthermore, of approximately 16,000 *S. vulgaris* progeny raised from seed collected at the same sites, two were determined to be the triploid hybrid. It is evident from these records that interspecific hybridization between *S. squalidus* and *S. vulgaris* occurs regularly, although at low frequency in the wild.

Introduction and spread of *Senecio squalidus*

The spread of *S. squalidus* in the British Isles has been well documented and noted to coincide with the origin of a number of hybrid taxa (Crisp, 1972). The species is thought to have been introduced first to Britain to the Oxford Botanic Gardens following collections made in Sicily before 1699 (Druce, 1927; Kent, 1956; Crisp, 1972). Unfortunately the Gardens' records from this period were lost in a fire, so an exact date of introduction and the source location of the introduced material remain unclear (Crisp, 1972). Recent morphological (Crisp, 1972; Parry-James, 1995), isozyme (Parry-James, 1995) and cpDNA (Abbott, Curnow and Irwin, 1995) evidence strongly suggest that British *S. squalidus* is derived from material collected from a hybrid swarm between *S. aetnensis* and *S. chrysanthemifolius,* found on the slopes of Mount Etna, Sicily, rather than from southern continental *S. squalidus* material, often referred to by continental taxonomists as *S. rupestris* (Alexander, 1979). *S. squalidus* was first described by Linnaeus in 1753 from material cultivated at Uppsala and said to be a southern European annual (Crisp, 1972). The material was believed to have been supplied by J.J. Dillenius, the first Sherardian Professor of Botany at Oxford (Walker 1833, in Kent 1956); however, this claim was disputed by Smith (1828, in Kent 1956) who suggested that there was no proof for it. Crisp (1972) undertook a comparison of the Sicilian and British taxa, and found that the original material used for Linnaeus' description was very similar to that presently found in Britain. After its introduction, *S. squalidus* was cultivated in the Oxford Botanic Gardens for nearly 100 years until 1792 when a specimen 'escaped' and was found on a wall outside the Garden in Oxford (Druce 1927; Crisp, 1972). Early dispersal from Oxford is thought to have been helped considerably following deliberate introductions by interested naturalists and was further aided by the development of the railway system (Kent, 1955; 1960). The plumed-seeds of *S. squalidus* are light and easily carried in the vortex of air following express trains, as witnessed by Druce (1927; Kent, 1960). Later spread followed industrial materials shipment (Kent, 1960) and road development. In the latter half of this century the species has become widespread in the British Isles on

walls, roadsides, railways, waste sites (Abbott, 1992) and in gardens (Tutin, 1973). The spread of *S. squalidus* from the Oxford Botanic Gardens has been well documented by Kent (1954-5; 1956; 1960; 1962-4; 1963; 1964a; 1964b; 1964c; 1964d and 1966) and summarised by Crisp (1972).

Soon after the spread of *S. squalidus* into some areas of the British Isles, hybrids and hybrid derivatives were reported, including *S.* x *baxteri* (Crackles, 1990), *S. vulgaris* var. *radiatus* (Lousley, 1944, Crackles, 1990), *S. cambrensis* (Rosser, 1955) and semi-fertile tetraploid hybrids (in Crisp, 1972). Such hybrids and hybrid products were found mainly in areas where *S. squalidus* and *S. vulgaris* formed large mixed populations. During and after the second world war, bomb sites provided large disturbed areas for colonization by these two species (Lousley, 1943 and 1944; Kent, 1964c). For example, by 1950 *S. squalidus* was recorded on up to 62% of bomb sites and other waste places in and around Hull (Crackles, 1990). It was speculated that *S. squalidus* did well on these sites due to habitat similarities between the burned ground of bomb sites and the soils found on Mount Etna (Kent, 1956). The similarity between the clinker ash substrate used for railway sleeper stabilization and soils has also been offered as a reason to explain the rapid spread of *S. squalidus* through the railway network (Druce, 1927; Ashton, 1990).

Origin of *S. cambrensis*

The newly evolved allopolyploid, *S. cambrensis*, was first described by Rosser (1955) from a specimen discovered in 1948 by H.E. Green at Cefn-y-Bedd, North Wales. The plant is now commonly found in Wrexham and the surrounding area, and has been reported from Mochdre, near Colwyn Bay, North Wales, in 1966 (Brumitt 1971) and from Edinburgh, Scotland, in 1982 (Abbott, Ingram and Noltie, 1983). The species has maintained itself at all of these sites; however, recent development of the derelict habitats in Leith has lead to a sharp decline in the number of individuals in Edinburgh (personal observation). *S. cambrensis* is a hexaploid (2n=60), thought to have arisen following chromosome doubling of the triploid hybrid *S.* x *baxteri* (Rosser, 1955; Weir and Ingram, 1980). There is good molecular evidence for at least two independent origins of *S. cambrensis* in the British Isles, one in North Wales and the other in Edinburgh (Ashton and Abbott, 1992; Harris and Ingram, 1992a; Lowe and Abbott, 1996).

Origin of the stabilized introgressant, *S. vulgaris* var. *hibernicus*

Early records

Inland, radiate forms of *S. vulgaris* in the British Isles were first recorded in the 19th Century, and some taxonomists suspected them of being the products of introgression of *S. squalidus* genes into *S. vulgaris*. The first confirmed record of radiate groundsel was from Oxford in 1832 (Crisp, 1972), but Syme's (1875) description of *S. vulgaris* var. *hibernicus* was not until 1866, and was made on material growing profusely around Cork, in Ireland. Before the end of the 19th Century *S. vulgaris* var. *hibernicus* was recorded as well established in four areas; southern Ireland; Oxford; Bristol/Cardiff and North West Wales/Cheshire. It subsequently extended its range greatly in the first part of the 20th Century. Crisp (1972) reported the dates of the first vice county records for *S. vulgaris* var. *hibernicus* during its spread in the British Isles and noted that these were strongly correlated with the dates of first vice county records for *S. squalidus*. This indicated that inland radiate groundsel appeared in an area soon after *S. squalidus* had become established in the same area.

S. vulgaris var. *hibernicus* is easily distinguished from non-radiate *S. vulgaris* var. *vulgaris*, by the presence of an outer whorl of approximately 8-13 ray florets in its capitula. Early records of var. *hibernicus* were often confused with the triploid hybrid, *S.* x *baxteri,* and the ancient coastal radiate form, *S. vulgaris* ssp. *denticulatus*. Allen (1967) referred initially to all fertile rayed forms of *S. vulgaris* as var. *radiatus* Koch, and this name is still widely used (Crisp, 1972). However, Allen (1967) subsequently clarified the taxonomic situation by naming the hirsute, shortly ligulate coastal form as var. *denticulatus* (O.F.Muell) Hyland, and the inland form as var. *hibernicus* Syme. Later Sell (1967) elevated *denticulatus* (O.F.Muell) P.D. Sell to sub-specific level and reduced *hibernicus* to formal status, f. *ligulatus* D.E. Allen, although inland radiate groundsel remains more commonly referred to as var. *hibernicus* Syme (Crisp, 1972; Stace, 1991).

Trow's 'microspecies'

Trow (1912) recognized the close affinities between non-radiate and radiate forms of *S. vulgaris*. He further bred a number of pure *S. vulgaris* lines distinguished by heritable differences in the presence or absence of ray florets, ray floret colour, hairiness, stem colour, leaf colour and number and distribution of nodes along the main axis (Trow, 1912, 1916). The genetic control of some of these characters was tested and the ray floret character was found to be controlled by a single locus expressing alleles either promoting the presence of ray florets, T_r, or the absence of ray florets, T_n. The alleles were found to be codominant and the heterozygote, T_rT_n, expressed

rays of an intermediate length (Trow, 1912). Trow proposed that his pure bred lines should be used to acknowledge several new 'microspecies' of *S. vulgaris*, but admitted that most could not be easily differentiated in the wild (Trow, 1912). However, what Trow referred to as *S. lanuginosus* was originally collected from Jersey and is undoubtedly *S. vulgaris* ssp. *denticulatus*. Another of his microspecies, *S. erectus*, was noted as the most common form of *S. vulgaris* and occurred as radiate (var. *radiatus*) and non radiate forms. Trow separately recognized *S. latifolius* as similar to *S. erectus*, but more robust, while *S. multicaulus* was characterized by the production of many branches from the basal axils. This later taxonomic label has been used to describe a number of herbarium specimens displaying such character (personal observation). Another of his 'microspecies', *S. genevensis*, appears more typical of continental European *S. vulgaris* material (H.P. Comes, personal communication), while *S. praecox* had a rosette growth habit similar to that observed in coastal forms. Although Trow established the genetics of the radiate condition in *S. vulgaris* he never discussed its possible introgressive origin.

Early evidence for the introgressive origin of *S .vulgaris* var. *hibernicus*

Several papers were published in the 1970's on the possible introgressive origin of radiate groundsel. Richards (1975) observed that British radiate groundsel showed markedly slower growth characteristics than the non-radiate form, and was in fact more similar in this respect to a population of non-radiate groundsel from Yugoslavia. Richards further speculated that the slow growth of British radiate groundsel may have been inherited from *S. squalidus* as part of a linked gene complex that had been introgressed with the ray floret locus into *S. vulgaris* from *S. squalidus*. Monaghan and Hull (1976) further demonstrated that a population of radiate *S. vulgaris* plants from Edinburgh, where *S. squalidus* was common, possessed leaf dimensions more similar to *S. squalidus* than non-radiate plants from a Glasgow population, where *S. squalidus* was absent. Monaghan and Hull speculated that the observed morphological differences between *S. vulgaris* populations was due to introgression of *S. squalidus* genes for leaf shape into Edinburgh populations of *S. vulgaris*. Hull (1974b) had previously recorded differences in the distribution of esterase variants between a monomorphic population of non-radiate groundsel and the mixed groundsel populations at Edinburgh and Glasgow, and suggested that these differences were the result of continued gene flow from *S. squalidus* into *S. vulgaris*. However, he found no significant difference in esterase distribution between radiate and non-radiate plants within or between polymorphic sites. In another study, Hull (1975) examined the relative frequency of alleles controlling the ray floret locus in mixed populations of *S. vulgaris*, he found that there was a significant increase in the frequency of the *Tr* allele

in populations where plants of *S. squalidus* were also common, relative to groundsel populations where *S. squalidus* was rare or absent. Hull favoured the hypothesis that the radiate form was maintained in the wild by repeated introgression from *S. squalidus* rather than because of differences in fitness between the groundsel morphs (Hull, 1976). This hypothesis was disputed by Oxford and Andrews (1977), who claimed that fitness differences between morphs were of a type that could maintain the radiate morph.

Stace's mutation hypothesis

Even though evidence seemed to be mounting to support the idea that *S. vulgaris* var. *hibernicus* was a product of introgressive hybridization between *S. squalidus* and *S. vulgaris*, not all workers were in agreement. Stace (1977) pointed out that there was only 'weak circumstantial evidence' to support the hypothesis and proposed that it was equally likely that radiate groundsel arose as a result of a single mutation causing ray florets to be produced in capitula. Such mutations had been recorded in other members of the Asteraceae. Stace went on to question the lines of evidence that had been used to support the introgression hypothesis. He pointed out that, although the first vice county records of *S. vulgaris* var. *hibernicus* broadly followed those of *S. squalidus*, there were several discrepancies. Firstly, there were occasions when *S. vulgaris* var. *hibernicus* had been recorded in a vice county before *S. squalidus* (most notably in Northern Ireland). Secondly *S. squalidus* had been common in London since 1940; however, up to 1952, *S. vulgaris* var. *hibernicus* had been recorded in the area only six times, and remains rare to the present time. Thirdly, the triploid hybrid, *S.* x *baxteri*, was very rare and there was no experimental evidence to demonstrate that it produced fertile progeny. Fourthly, the claim by some workers that *S. vulgaris* var. *hibernicus* possessed a morphology more similar to *S. squalidus* than to var. *vulgaris* was simply not true, and the only real difference between the two variants of *S. vulgaris* was the presence of ray florets.

Recent evidence for the introgressive origin of *S. vulgaris* var. *hibernicus*

Stronger evidence to support the introgression hypothesis for the origin of *S. vulgaris* var. *hibernicus* emerged from additional isozyme analysis. Abbott, Ashton and Forbes, (1992) reported that an allele encoding an allozyme of AAT (i.e. *Aat*-3*c*), which occurred at high frequency in British *S. squalidus* populations, was present at an intermediate frequency in populations of *S. vulgaris* var. *hibernicus*, but was absent from populations monomorphic for var. *vulgaris*. Further genetic analysis showed that this isozyme locus segregated independently from the ray floret locus, providing good

evidence that genes on more than one chromosome have been introgressed into *S. vulgaris* from *S. squalidus* and have tended to remain associated in var. *hibernicus*.

Origin of York radiate groundsel, a new tetraploid hybrid

Irwin and Abbott (1992) proposed that hybridization between *S. vulgaris* and *S. squalidus* has also led to the formation of York radiate groundsel, a fertile tetraploid. This hybrid product exhibits an intermediate morphological phenotype, and possesses additive esterase and RAPD profiles that combine the divergent phenotypes of the parental taxa (Chapter 2; Irwin and Abbott 1992). These characters clearly distinguish York radiate groundsel individuals from standard inland radiate groundsel, *S. vulgaris* var. *hibernicus*.

Radiate groundsel has been recorded in the York area only in recent times, although *S. squalidus* was first reported in the East Ridings area at Hull Docks in 1926 (Kent, 1960; Crackles, 1990) and by the end of the second world war had become widely established in the surrounding area (Kent, 1964). The first record of *S. squalidus* near the city of York was made by E.J. Payne at Acomb on 2. 5. 1938 (York and District Field Naturalists Society, T. Medd, personal communication). It was not recorded again until 1948 when it was seen by K.G. Payne at Tadcaster Road (T. Medd, personal communication), but by 1957 had become widespread in York (Kent, 1964). *S. vulgaris* var. *hibernicus* was first recorded from Hull in December 1950 and is now an abundant weed in the Hull Botanic Gardens (Crackles, 1990). In York, the first record of rayed groundsel was made on 11. 8. 1958 from under a beech hedge on the banks of the Ouse, South West of Lendal Bridge (T. Medd, personal communication). A population of rayed groundsel was also seen by T. Medd on a building site at Acomb Road on 11. 6. 1960. As a detailed description of these specimens is lacking, it is not possible to determine whether these reports refer to var. *hibernicus* or York radiate groundsel. York radiate groundsel was first noted as different from var. *hibernicus* when it was collected in 1979 from the edge of a car park near York railway station by R.J. Abbott and D.F. Marshall. Since then, this hybrid derivative has been recorded at several sites in and around York (Figure 3.2). *S. vulgaris* var. *hibernicus* is also found in York (see Figure 3.2), but the two forms of radiate groundsel do not appear to form sympatric populations (J. Warren, personal communication). Increased weeding and redevelopment of the Dalton Terrace and Lendal Bridge sites during the period of the current research project has tended to reduce population sizes of York radiate groundsel at these two locations.

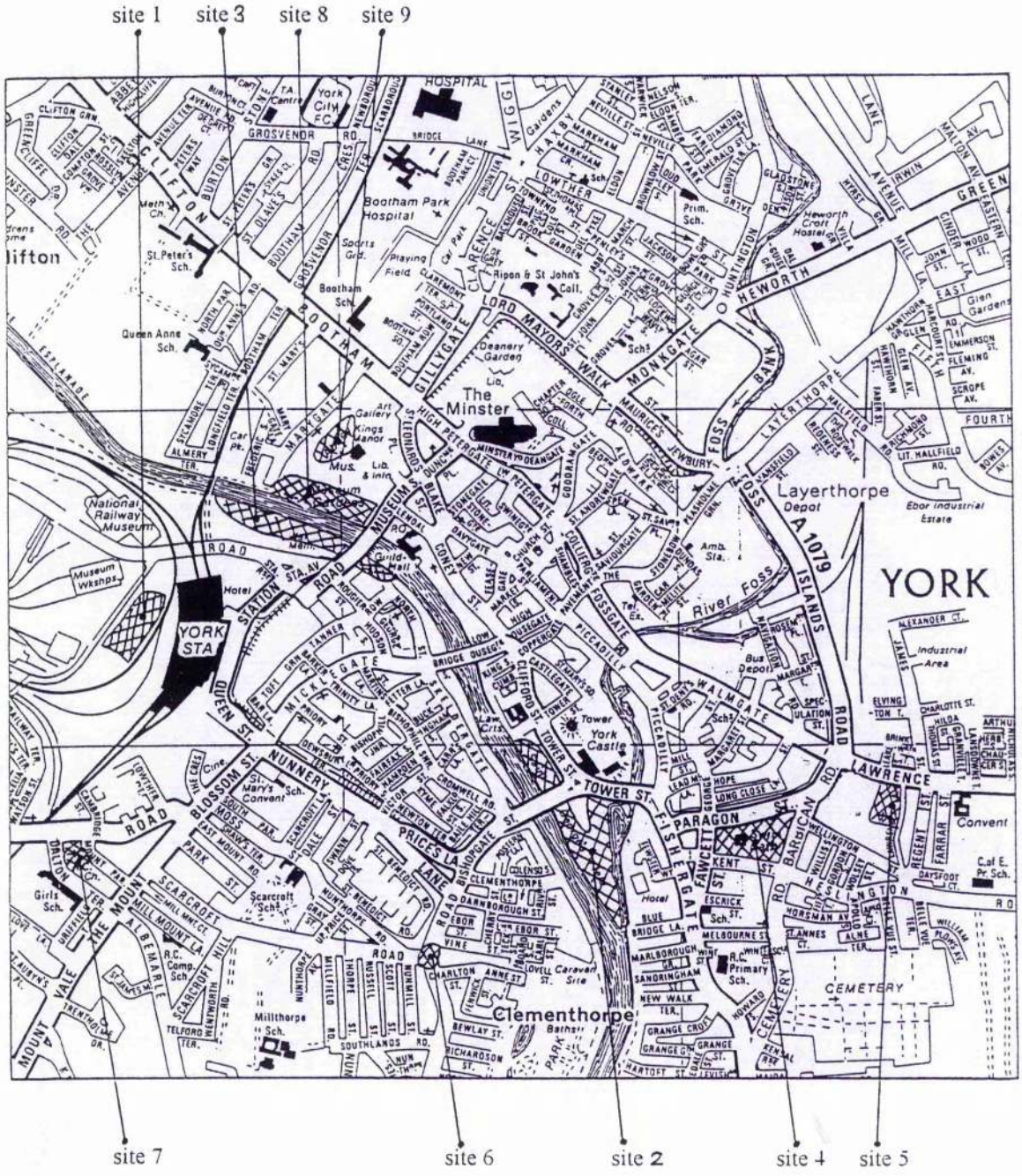


Figure 3.2. Reconstruction of past distribution of radiate groundsel populations around the centre of York (R.J. Abbott, T. Crawford and J. Warren, personal communication). Site 1, car park near York railway station, found 1979; site 2, along the river Ouse East downstream of Lendal Bridge, early/mid 1980s lost to river side development in late 1980s; site 3, river bank at Lendal Bridge, early/mid 1980s until present; site 4, area around Barbican centre, early/mid 1980s until late 1980s when lost to leisure centre development; site 5, grounds of church on Lawrence Street, mid 1980s until early 1990s; site 6, Bishopthorpe Road roundabout construction, late 1980s; site 7, car park of church on Dalton Terrace, early 1990s until present. *S. vulgaris* var. *hibernicus* individuals distinct from York radiate groundsel individuals were noted on the north bank of the river Ouse west of Lendal Bridge, at site 8, in the early 1990s; and around Kings Manor, site 9, in mid/late 1980s

Herbarium evidence for fertile intermediate hybrids similar to York radiate groundsel

York radiate groundsel possesses a number of novel morphological features that easily distinguish it from *S. vulgaris* var. *vulgaris,* var. *hibernicus* and *S. squalidus.* These features, which include long achenes (>2.5 mm), four pored pollen, and long, many lobed leaves and 'showy' capitula, are easily recognized in herbarium specimens. However, some of these characters are also exhibited by the hexaploid hybrid, *S. cambrensis,* but York radiate groundsel can be distinguished from the latter by the possession of longer, less dissected, more lobate leaves, narrower capitula and eight rather than 13 ray florets. According to Benoit, Crisp and Jones, (1975), hybrid swarms between *S. vulgaris* and *S. squalidus* have been found in at least 20 English and Welsh vice-counties and in three Irish vice-counties. These are described as probably tetraploid and distinct from *S. vulgaris* var. *hibernicus.* They have been found on waste ground, disturbed soils, railway embankments and similar habitats. Benoit, Crisp and Jones, (1975) reported that some plants in the tetraploid hybrid swarms had "..lower seed- and pollen-fertility than the parent species, but fertile achenes are often larger, and stainable pollen-grains often have four rather than three pores." G.C. Druce also recognized that some hybrid plants, similar to those described by Benoit, Crisp and Jones, were sufficiently distinct from other taxa to loosely attach the name *S. advena* to them (Crisp, 1972; personal observation of herbarium specimens), although the name was never officially registered and no type specimen exists (*Index Kew.*). In a survey of British herbarium material, Crisp (1972) listed 46 individuals that, in his experience, were fertile or partially fertile hybrids between *S. vulgaris* and *S. squalidus,* and distinct from var. *hibernicus.* Crisp based his identification of hybrid herbarium specimens on a number of characters, amongst them; partial or full fertility, large capitula, long achenes and ragged or dissected leaf shape. From his notes, it is clear that a large number of these hybrids had been misclassified previously as *S. vulgaris* var. *radiatus, S. x baxteri, S. nebrodensis, S. vernalis* and even forms of *S. squalidus.* Crisp (1972) noted that many of the hybrids were collected along with other material from a hybrid swarm. Some specimens showed signs of backcrossing to *S. vulgaris* and a range of material was often present from intermediate hybrids, through to specimens indistinguishable from *S. vulgaris* var. *hibernicus,* Crisp gave the label 'introgression sequence' to such material.

From a personal examination of material supplied by ten herbaria (mentioned previously) plus the notes of Crisp (1972), I was able to identify partially fertile (possibly tetraploid) hybrids with long achenes (>2.5 mm), highly dissected leaves and large capitula. It was also possible to identify tentative backcrosses between intermediate hybrids and *S. vulgaris.* Backcross individuals were generally similar in

morphology to *S. vulgaris*, whilst still retaining some hybrid characters e.g. large capitula, ray florets, long achenes or distinctive leaf shape. Forty intermediate hybrids and 36 backcross individuals were identified in this way (Table 3.2). Some of the fertile intermediate hybrids were very similar in appearance to York radiate groundsel (see Figure 3.3a, b, c and d for specimens of York radiate groundsel, putative intermediate hybrid, putative progeny of intermediate hybrid backcrossed to *S. vulgaris* and *S. vulgaris* var. *hibernicus*). Such intermediate hybrids were mainly found among specimens collected from four areas (Cork, Bristol/Cardiff, Wrexham/Liverpool and Oxford), and most of the backcross products were also collected from these locations.

Studies and recent field observations of fertile intermediate hybrids similar to York radiate groundsel

Crisp (1972) discovered a single hybrid plant, referred to as S602, on a waste site in London in November 1966. This short-rayed plant resembled *S. cambrensis* in a number of morphological features, including leaf shape, ligule length (6.7 mm, 13 ligules), pollen pore number (3 and 4), pollen diameter (30.2 μm) and achene length (3.3 mm). Unfortunately the plant died before a chromosome count could be made, but nearly 100 field pollinated progeny were raised and all were more or less tetraploid (2n=40). The S602 progeny segregated for characters that distinguished *S. vulgaris* and *S. squalidus* including; mildew susceptibility (common in *S. vulgaris*), leaf shape and size (although none produced typical *S. squalidus* or *S. vulgaris* leaves) and ray floret length. Progeny also tended to be polycarpic (typical of *S. squalidus*) rather than monocarpic (typical of *S. vulgaris*). On average, S602 progeny exhibited similar pollen stainability (76.9%) and seed set (42.4%) to their parent (83.4 and 68.2% respectively); however, S602 progeny exhibited a wide range of fertility (pollen stainability, 3.3-93.9%; seed set, 0.2-95.6%). Other characters that occur in hybrids also segregated in the S602 progeny. These were achene length, which tended to decrease from 3.5 mm to 2.4-2.9 mm, and pollen diameter and pore number which varied among segregating offspring. From the descriptions and drawings of leaf shape in Crisp's thesis, it is evident that some of the S602 progeny were very similar to York radiate groundsel in appearance. Backcrosses between some of the fertile, radiate S602-progeny and *S. vulgaris*, produced highly fertile offspring (with pollen stainability greater than 95% and average seed set greater than 59% and up to 73%) which appeared similar to var. *hibernicus* in ray floret and capitulum dimensions and leaf shape.

Table 3.2. List of putative specimens of partially or fully fertile hybrids (hybrid) between *S. squalidus* and *S. vulgaris* and backcrosses to *S. vulgaris* (backcross) identified from herbaria at Reading (RNG), Kew (K), Cambridge (CGE), British Museum (BM), Britol Museum (BRISTM), Leicester (LTR), Liverpool University (LIVU), Irish National Herbarium (DBN), Manchester (MANCH) and Oxford (OXF). Specimens include those identified only by Crisp (1972) and references can be found therein. Specimens identified by P. Crisp and seen by me are acknowledged in the comments. The fertile intermediate hybrids were identified by the possession of long achenes (>2.5 mm, see achenes, all lengths in mm), some possibly being abortive (sterile), and an intermediate hybrid leaf pattern (see leaf; h=hybrid). Backcrosses between the fertile tetraploid intermediates and *S. vulgaris* showed characters more similar to *S. vulgaris* while still retaining some hybrid characters eg. long achenes or distinctive leaf shape (see leaf; v=*S. vulgaris* leaf; r=*S. vulgaris* var. *hibernicus* leaf; h=hybrid leaf; c=*S. cambrensis* leaf; sq=*S. squalidus* leaf). Specimens are arranged in location alphabetical order.

Taxa	Year	Locality	Source	Achenes	Leaf	Comments
backcross	1939	Aberdovey	LTR	fert. 3	v(r)	'Possibly *S.vulgaris* var. *radiatus*, but could be *S.crassifolius* Willd' H.H.Haines.
hybrid	1894	Barmouth	K			'leaves resembling *S.cambrensis*, heads large other wise resembling *hibernicus*' Crisp 1972
backcross	1930	Bristol	BRISTM	fertile	v	I.W.Evans
backcross	1945	Bristol	LTR	?	h	'Short, tubular rays'. 'too young for examination of achenes, maybe *S.squalidus*x*vulgaris*'-NFS
hybrid	1945	Bristol	LTR	fert. 2.2	h	Gibbons & Bell;'Rather strongly ribbed globescent achenes, I believe *S.squal.* x *vulg.*'-NFS
hybrid	1945	Bristol	LTR	fert. 2	h(s)	'Thick ribbed or globescent achenes. This is surely *S.squalidus* x *vulgaris*'-NFS
backcross	1946	Bristol	LTR	fert. 3	h	Gibbons & Bell '*S.vulgaris* var. *radiatus* I believe, achenes seem to be those of *vulgaris*'-NJS
backcross	1948	Bristol	BRISTM	fert. 3	h	'*S.squalidus* x *vulgaris*'; some fertility, short rays, backcross to *S.vulgaris*?
backcross	1909	Bristol, Portishead	LTR	fert. 2.5	v(r)	I.M.Roper.Also secimen at BIRM 'resembles large specimen of *hibernicus*, seeds large' P.Crisp
backcross	1917	Bristol, Portishead	BM			'resembles var. *hibernicus*, but with large seeds' Crisp 1972
hybrid	1930	Burton-on-Trent	OXF	fertile		'*S.advena*' Druce; 'An S602 type' Crisp 1972
hybrid	1900	Cardiff	K	fertile		'Recorded as x *baxteri*, which it resembles, but seed is set' Crisp 1972
backcross	1905	Cardiff	LIV	fertile	h	'*S.vulgaris* x *squalidus*' H.J.Riddelsdell; 'introgression sequence' Crisp 1972
hybrid	1905	Cardiff	CGE	part fert.		'achenes abortive' Rosser; 'pollen large, some 4-pored, low stainability; ligules short and broad;
						'leaves resemble squalidus type. Definately S602 type; Crisp 1972; RBEC (1906):228
backcross	1906	Cardiff	LIV	fertile	h	'*S.vulgaris* x *squalidus*' H.J.Riddelsdell 'introgression sequence' Crisp 1972; *RBEC* (1906):228
backcross	1906	Cardiff	LIV	fertile	h	'*S.vulgaris* x *squalidus*' H.J.Riddelsdell 'introgression sequence' Crisp 1972; *RBEC* (1906):228
hybrid	1906	Cardiff	BM	fert. 3	h	'*S.vernalis*' H.J.Riddlesdell.ref 30574; 'Another S602 type' Crisp 1972; *RBEC* (1906):228
hybrid	1908	Cardiff	BM			'*S.nebrodensis*' Riddelsdell; 'two typical S602 types, one with leaves nearer to *vulgaris*' Crisp 1972
hybrid	1927	Cardiff, Barry	Crisp 72	fertile		'*S.vulgaris* x *S.vernalis*' C.G.Druce; *RBEC* (1927):401
hybrid	1984	Cardiff, Bridgend	LTR	fert. 2.2	v(r)	'this is hybrid *S.vulgaris* x *squalidus*' R.P.Libbey
backcross	1910	Cardiff, Llandoff	BM	fert. 2.5	h/r	'*S.vulgaris*, short rays' H.J.Riddlesdell; ref 30556
backcross	1912	Cardiff, Llandoff	BM	fert. 2.5	h/r	'*S.vulgaris*' H.J.Riddlesdell; ref 30563
backcross	1912	Cardiff, Llandoff	BM	fert. 2.5	h/r	'*S.vulgaris*' H.J.Riddlesdell; ref 30562
backcross	1912	Cardiff, Llandoff	BM	fert. 2.5	h/r	'*S.vulgaris* var. *radiatus*' H.J.Riddlesdell; ref 30555
hybrid	1912	Cardiff, Llandoff	BM	fert. 2.5	h	'*S.vulgaris*' H.J.Riddlesdell; ref 30565
backcross	1943	Cardiff, Llandoff	BM	fert ?	h	'*S.squalidus* x *vulgaris*' A.H.Alston; Ref 30183
backcross	1819	Cork	K			'Old specimen on which the date is not clear; resembles an S602/*hibernicus* int.' Crisp 1972
hybrid	1853	Cork	CGE	fertile		'Heads fairly large, otherwise intermediate, seeds set' Crisp 1972
backcross	1861	Cork	DBN	fert 2.5	r/h	'Intermediate in the introgression sequence *vulg*x*squal* to *hibernicus*' Crisp 1972; Acute thin leaf
backcross	1867	Cork	MANCH			'Nearer *hibernicus* than S602, pollen regular and fairly small' Crisp 1972

Table 3.2. Continued.

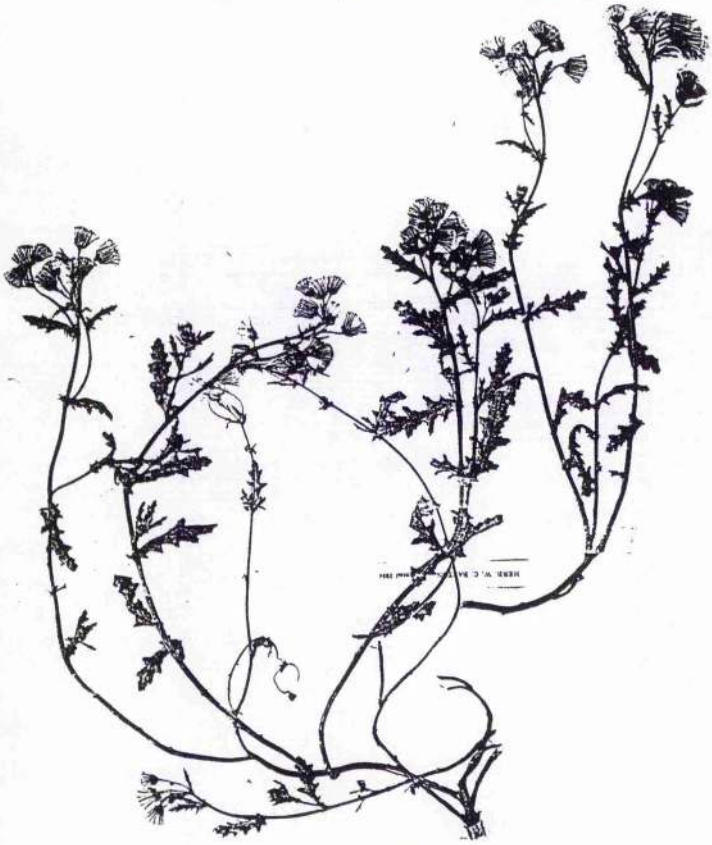
backcross	1878	Cork	Crisp 72			*'S.vernalis'* Boswell; 'var. *radiatus'* E.Rosse;r 'Probably S602/*hibernicus* intermediate' Crisp 1972
backcross	1879	Cork	MANCH			'An S602/*hibernicus* type; pollen large, mostly 3-pored, some unstained' Crisp 1972
backcross	1888	Cork	DBN			'Large heads, broad ligules, seed fairly large; otherwise resembles *S.vulgaris'* Crisp 1972
hybrid	1895	Cork	DBN	fert 2.5	h	R.A.Phillips; Both specimens hybrids of some description
hybrid	1896	Cork	DBN	fert. 2.5	v	R.A.Phillips; Right specimen *squalidus*, left one fertile hybrid
backcross	1899	Cork	DBN	fert 2	v(r)	'a late intermediate in the introgression sequence *vulgxsqual* to *hibernicus'* Crisp 1972
backcross	1901	Cork	K	fert 2.2	h/v	weed of horticulture at Cambridge Botanic Gardens A.Hosking
backcross	1902	Cork	DBN	fert. 2	v(r)	'an intermediate in the introgression sequence *vulgxsqual* to *hibernicus'* Crisp 1972
hybrid	1903	Cork	DBN	par. fert. 2	h/r	'Grown in garden from field seed' H.L.Colgan
backcross	1904	Cork	DBN	fert 2.2	h	'int.*S.vulgxsqual/hibernicus* backcross sequence or even *S.cambrensis*, large pollen' Crisp 1972
hybrid	1894	Cork, Greystones	DBN			'Large plant, heads and achenes with int. ligules. leaves near *S.squalidus*.Prob. S602' Crisp 1972
backcross	1901	Cork,PassageWest	DBN	fert. 2.5	y	'an intermediate in the introgression sequence *vulgxsqual* to *hibernicus'* Crisp 1972
backcross	1907	Cork,PassageWest	DBN	fert. 2.7	r/h	'intermediate in the introgression sequence *vulgxsqual* to *hibernicus'* Crisp 1972; acute thin leaf
backcross	1977	Dorset	RNG	fert. 2.5	v(r)	*'S.squalidus* x *vulgaris'* H.J.M.Bowen. leaves *vulgaris* like, slightly elongated, long ligules
backcross	1956	Exmouth	RNG	fert 2.3	v(r)	*'S.squalidus* x *vulgaris'* V.M.Wilkinson; leaves ragged but like *S.vulgaris.*
backcross	1956	Exmouth	LIV	fertile	v	*'S.squalidus* x *vulgaris'* J.E.Lousley
backcross	1971	Kings Lynn	LTR	fert. 2.2	v(r)	collected as *S.squalidus* x *vulgaris*-R.P.Libbey; But more probably var. *hibernicus*
hybrid	1972	Kings Lynn	LTR	fert. 2.5	thin	*S.squalidus* x *vulgaris*-R.P.Libbey
hybrid	1972	Kings Lynn	LTR	fert. 2	h	*S.squalidus* x *vulgaris*-R.P.Libbey
hybrid	1973	Kings Lynn	LTR	fert. 2.4	v(r)	*S.squalidus* x *vulgaris*-R.P.Libbey
backcross	1974	Kings Lynn	LTR	fert. 2	v(r)	*S.squalidus* x *vulgaris*-R.P.Libbey; Some intemediate leaf characters, probably backcross.
backcross	1974	Kings Lynn	LTR	fert. 2	v(r)	*S.squalidus* x *vulgaris*-R.P.Libbey; Introgression intermediate Crisp 1972
hybrid	1976	Liverpool	LIV	some fert.	h	*'S.squalidus* x *vulgaris'* A.J.Coombes
hybrid	1977	Liverpool	LIV	fertile	h	*'S.squalidus* x *vulgaris'* A.J.Coombes; could be S.*cambrensis*
hybrid	1977	Liverpool	LIV	fertile	h	*'S.squalidus* x *vulgaris'* A.J.Coombes; could be S.*cambrensis*
backcross	1946	Muirehead	LTR	fert. 2	v(r)	from J.E.Lousley's garden; 'this is advanced backcross product' Crisp 1972
hybrid	1962	Oldham	K	2-5% 2.7	h/a	*'S.xbaxterii'*. C.E.Shaw; leaf apex acutely angled, 2-5% seed set, certainly hybrid intemediate
hybrid	1887	Oxford	Crisp 72			'a rare form found around Oxford resembling *S. crassifolius'* G.C.Druce; *RBEC* (1887):184.
hybrid	1915	Oxford, Botley	Crisp 72			'near *S. crassifolius'* Druce; 'large *praecox* type' A.H.Trow; *RBEC*:(1915):352
hybrid	1929	Oxford, Didcot	BM	fert	h	*'S.advena'* Druce; Crisp 1972
hybrid	1945	Parkstone, Dorset	BM	fert. 3	h	*'S.vulgaris* x *Ssqualidus'*J.E.Lousley; large capitula, dissected leaves; ref 30483
hybrid	1945	Plymouth	RNG	fert. 3	r	*'S.squalidus*x*vulgaris'*E.Masson; large capitula, *S.vulgaris* type leaves

Table 3.2. Continued.

hybrid	1903	Reading	Crisp 72	fertile		'discussed by Druce and W.O.Focke who noted hybrid character' Crisp 1972; *RBEC* (1903)
hybrid	1890	Shelwith Bridge	BRISTM	fertile		'heads in dense cluster; ligules short and broad; seeds large; pollen large and regular' Crisp 1972
hybrid	1974	Strathclyde Uni.	LTR	fert. 2	h	*S.vulg.* x *squal.* introgressant (long ligulate) from garden of Hull; *Wat.* (1974).**10**.1:66-75.
hybrid	1974	Strathclyde Uni.	LTR	fert. 2	h	*S.vulg.* x *squal.* introgressant (int. ligulate) from garden of Hull; *Wat.* (1974).**10**.1:66-75.
hybrid	1974	Strathclyde Uni.	LTR	fert. 2	h	*S.vulg.* x *squal.* introgressant (short/nil ligulate) from garden of Hull; *Wat.* (1974).**10**.1:66-75.
backcross	1928	Surrey	K	fert	v/r	*'S. erectus* var *radiatus'* A.H.Trow
hybrid	1956	Unstone, Derbys	LIV	some fert.	r/h	*'S.vulgaris* x *squalidus'* H.J.Riddelsdell
hybrid	1927	Wrexham	MANCH			*'squalidus* leaves; large, stainable, 4-pored pollen. Could be S602 or *S.cambrensis'* Crisp 1972
hybrid	1948	Wrexham, Cefn-y-B	BM	fert. 2.5	h/v	*'S.cambrensis'* E.P.A.Jones; ref 30448
hybrid	1948	Wrexham, Denbigh	BM	fert. 3	h	*'S.squalidus* ?' ref 30278
hybrid	1948	Wrexham, Ffrith	BM	fertile	h/c	*'S.cambrensis'* H.E.Green; ref 30379; could be hybrid or *S.cambrensis* x *vulgaris*
hybrid	1978	Wrexham, Ffrith	LIV	fertile	h	*'S.cambrensis'* I.D.Wallce; probably hybrid
hybrid	1977	Wrexham, Mold	LIV	fertile	h	*'S.squalidus* x *vulgaris'* A.J.Coombes; could be S.*cambrensis*
backcross	1882	Yarmouth	BM	fert. 3	v	*'S.vulgaris* var. *radiatus'* E.F.Linton; wide basal lobes; Ref 30473

a. York radiate groundsel, showing dissected leaves, short rays, and intermediate capitula.

b. Hybrid between *S. vulgaris* and *S. squalidus*, (BRISTM) note the close similarity in leaf shape and capitulum dimension to York radiate groundsel.



c. Putative backcross between inter-mediate and *S. vulgaris* (BM). The plant still exhibits hybrid leaf characters, but also very short rays.

hybrid d. *S. vulgaris* var. *hibernicus* (RNG), showing leaf and capitula characters typical of var. *vulgaris*, but specimen exhibits ray florets.

Figure 3.3. Herbarium specimens of indicated taxa.

Crisp (1972) also reported finding two populations of unusual radiate groundsel individuals at Newbridge-on-Wye in Radnorshire in June 1967, and at Birmingham University Campus, Edgbaston, in October 1967. The Newbridge-on-Wye population was composed of plants with very long ligules (8.1 mm). This observation, together with an examination of ray floret length in the S602 progeny, lead Crisp to believe that a ligule length promoting gene may have been present and that there may be more than one gene involved in ligule development. At Edgbaston, plants possessed large capitula (like *S. squalidus*), highly dissected leaves, short ligules (6 mm long and 3 mm wide), long achenes (3-3.5 mm) and large, stainable pollen (80%). Radiate groundsel exhibiting characters similar to some of those of York radiate groundsel have more recently been reported by J. Warren (personal communication) near Temple Meads railway station, Bristol, in 1986, R. Milne (personal communication) at Avonmouth in 1991, and by S. Harris (personal communication) at Victoria train station, London, in 1992. R.J. Abbott (personal communication) reported the existence of a hybrid swarm between *S. squalidus* and *S. vulgaris* at Passage West, Cork in 1991, and noted that a wide range of leaf and capitulum variation was exhibited by plants at this location, with most plants exhibiting high seed fertility.

Most of the sites where hybrid individuals have been reported (either from herbarium specimens or personal communications), were visited by me between 1992 and 1994. These included sites at Avonmouth, Cork, Birmingham, Bristol, Cambridge, Edinburgh, London, Oxford and Wrexham. At none of these sites was an extant hybrid plant found, other than at Passage West, Cork, where a hybrid swarm still persisted. At this particular site, backcrossing to *S. vulgaris* appeared to be occurring with both hybrid and *S. vulgaris* extremes being present in the population. The most likely reason for the demise of hybrid populations/individuals at other sites seemed to be linked with habitat loss.

Experimental resynthesis of hybrids, a test of origin

The fact that products of hybridization between *S. squalidus* and *S. vulgaris* similar to York radiate groundsel have been observed from other locations suggests that occasionally hybrids of this type may be generated wherever the two parent species coexist. However, this observation does not give insights into the route of origin of these hybrids. The most effective way to test a proposed route of origin is by resynthesizing a hybrid product from an experimental cross. Previous studies that have resynthesized fertile hybrids have provided good tests for the evolutionary potential of certain hybridization events. In his classic work in 1930, Müntzing (in Briggs and Walters, 1984) tried to resynthesize the weedy species *Galeopsis tetrahit*

(2n = 4x = 32) by crossing two closely related diploid species (2n = 2x = 16), *G. pubescens* and *G. speciosa*. Highly sterile diploid F_1 hybrids were produced and selfed, and amongst the F_2 progeny was one triploid plant (2n = 3x = 24), presumably produced by the fusion of a reduced and unreduced gamete. Backcrossing the highly sterile triploid to *G. pubescens* produced a fertile tetraploid (2n = 4x = 32) which was morphologically similar to, and interfertile with, *G. tetrahit*. The tetraploid backcross was presumably produced following the fusion of an unreduced gamete of the triploid with a reduced gamete from *G. pubescens*.

Other resynthesis studies have been undertaken to demonstrate the evolutionary potential of hybridization, even though no fertile hybrid derivatives have been recorded in the wild. Work by Grant (1965, 1966), showed that fertile hybrid progeny could be obtained in later generations of a cross between *Gilia malior* and *G. modocensis*, both tetraploids in the *Gilia inconspicua* complex. The F_1 hybrid of this cross was nearly totally sterile; however, some F_2 seed was collected and raised to the F_{10}. One F_{10} plant was fertile and tetraploid; this hybrid was intermediate in morphology between its parents and produced sterile progeny when backcrossed to them, thus demonstrating a classic example of hybrid evolution by recombinational speciation (Grant, 1981). In another resynthesis experiment, Ratter (1972) demonstrated that fertile, true-breeding tetraploids could be generated in only two generations (F_3) from the highly sterile, triploid, interspecific hybrid between *Spergularia nicaeenis* (2n=4x=36) and *S. purpurea* (2n=2x=18). The fertile F_3 hybrids were mostly morphologically intermediate between the parental taxa, but also possessed some novel characters (very large flowers and rounded petals). When the F_3 was backcrossed to the tetraploid parent, *S. nicaeenis*, highly fertile progeny were produced which appeared similar to *S. nicaeenis*. Fertility in the F_2 was also high, and this resumption of fertility seemed to be associated with the ability of F_1 plants (2n=27) to produce progeny with great variation in chromosome number eg. 2n=24, 30 or 32. This made it possible for some plants to attain near tetraploidy in one generation and showed that there is considerable tolerance for chromosomal imbalance in this species (Ratter, 1973a). Cytological examination of the F_1 revealed that many trivalents were formed at meiosis, suggesting considerable genomic homology between *S. purpurea* and *S. nicaeenis* (Ratter, 1973b).

Although the ability to resynthesize a hybrid product in a particular way is powerful evidence for its route of origin, failure to produce a putative hybrid does not prove that hybridization cannot occur. For example, despite repeated attempts, the sterile hybrid *Spartina* x *townsendii* has never been generated from crosses between *S.*

alterniflora and *S. maritima*, nor has the allopolyploid, *S. anglica*, been synthesized by attempts to double the chromosome complement of *S.* x *townsendii* (Raybould *et al.*, 1991). However, from other lines of evidence, there remains little doubt that *S. alterniflora* and *S. maritima* are the parents of *S.* x *townsendii* and that chromosome doubling of the sterile hybrid gave rise to *S. anglica* (Gray, Marshall and Raybould, 1991).

Resynthesis of Senecio hybrids

Various workers have successfully resynthesized particular *Senecio* hybrids. For example, the hybrid between *S. sylvaticus* and *S. viscosus*, *S.* x *viscidulus*, has been synthesized with relative ease by Gibbs (1971), Crisp (1972), Taylor (1984) and Kadereit (1984), with pollen fertility of the hybrid ranging between 13.3-46.0% (Kadereit, 1984). Similarly the hybrid between *S. squalidus* and *S. viscosus*, *S.* x *subnebrodensis*, has been synthesized with relative ease by Crisp and Jones (1978) using *S. squalidus* as the maternal parent, and by Taylor (1984) using reciprocal crosses. Crisp and Jones (1978) reported that some seed was produced from this particular synthesized F_1 hybrid when left to open pollinate in a glasshouse and that the F_2 progeny were either triploid or approximately pentaploid and highly sterile (seed set 0.5%). However, the fertility of certain hybrid offspring increased markedly, as ploidy level moved first to sub-pentaploid (2n=48) in the F_3, to approximately tetraploid (2n=43-44) in the F_4 generation. Crisp and Jones (1978) were also successful in producing fertile hexaploid offspring (2n=60) following treatment of the F_1 hybrid with colchicine.

Comes and Kadereit (1990) have also synthesized the triploid hybrid, *S.* x *helwingii*, with relative ease, achieving greater success using *S. vulgaris* as the maternal parent. However, attempts to produce backcross offspring with either parent failed. The F_1 hybrid was highly sterile and produced no germinable seed when forced to self. Some seed was produced, however, when plants were left to open-pollinate in the glasshouse, and these seed developed into partially fertile, tetraploid or approximately hexaploid plants (both with a high level of pollen fertility, and seed fertility of 62.9 and 6.1 % respectively).

Although fertile progeny have been generated in later generations following hybridization between the above taxa, there are no records of such later generation fertile hybrids occurring in the wild (Benoit, Crisp and Jones, 1975; Comes and Kadereit, 1990; Crisp and Jones, 1978). Thus most of these hybridization events seem

to be evolutionary dead ends; however, this situation may partially reflect a lack of detailed investigation.

Resynthesis of *S.* x *baxteri*

The triploid hybrid, *S. x baxteri*, has been successfully synthesized by Harland (1954), Gibbs (1971), Ingram (1977), Ingram, Weir and Abbott (1980) and Taylor (1984), from crosses between *S. vulgaris* and *S. squalidus*. However, Crisp (1972) was unable to produce this hybrid, and Harland (1954) and Ingram (1977) only produced it when var. *hibernicus* was used as the *S. vulgaris* parent. More recently Ingram, Weir and Abbott (1980), produced *S.* x *baxteri* using either var. *vulgaris* or var. *hibernicus* as the maternal parent, while Taylor (1984) and Gibbs (1971) produced it with only var. *vulgaris* as the maternal parent. Normally, successful crosses are achieved only when *S. vulgaris* is used as the female parent, although Ingram (1977) successfully used *S. squalidus* as the maternal parent in one particular cross.

Resynthesis of *S. cambrensis*

The allohexaploid, *S. cambrensis*, has been artificially resynthesized by treating synthetic *S.* x *baxteri* individuals with colchicine (Harland, 1954; Rosser, 1955; Weir and Ingram, 1980; Ingram and Noltie, 1987). The resulting plants closely resembled wild ones in morphology and exhibited regular meiosis. Both synthesized and wild plants were self-compatible and showed high levels of seed set when left to self. Ingram and Noltie (1987) have backcrossed *S. cambrensis* to both parents, to produce a fertile pentaploid hybrid with *S. vulgaris* and a highly sterile tetraploid hybrid with *S. squalidus*. Despite numerous searches, neither of these backcross products have been found in the wild (Ingram and Noltie 1995).

Resynthesis of the stabilized introgressant, *S. vulgaris* var. *hibernicus*

Ingram, Weir and Abbott (1980) were also successful in backcrossing the triploid hybrid, *S.* x *baxteri*, from a cross between *S. vulgaris* var. *vulgaris* and *S. squalidus*, to *S. vulgaris*. The progeny were approximately tetraploid and fertile and some were similar in morphology to *S. vulgaris* var. *hibernicus*. Ingram (1978) had previously reported that the triploid hybrid of a cross between *S. vulgaris* var. *hibernicus* and *S. squalidus*, produced some offspring when left to open pollinate in a glasshouse. Some of these offspring were tetraploid, while others were diploid, pentaploid or hexaploid, but no morphological assessment was made of these hybrid products.

Resynthesis of partially fertile tetraploid hybrids between *S. vulgaris* and *S. squalidus*

In addition to synthesizing the triploid hybrid, Taylor (1984) synthesized a tetraploid F_1 hybrid from a cross between diploid *S. squalidus* and tetraploid *S. vulgaris*. This hybrid was presumably produced by fusion of a normal gamete of *S. vulgaris* with an unreduced gamete of *S. squalidus*. Taylor (1984) and Housten (1983) also synthesized a number of tetraploid F_1 hybrids by crossing *S. vulgaris* var. *vulgaris* with tetraploid *S. squalidus* plants (generated by colchicine treatment). The tetraploid hybrids produced by Taylor (1984) in either way were morphologically very similar to each other based on an analysis of 62 characters (Taylor, 1984). They produced large pollen (mean diameter 27.4 μm) with three or four pores, had long ligules (14.8 mm) and showed high pollen stainability. F_2 progeny of these hybrids exhibited a wide range of morphological variation, with individuals ranging in phenotype from those similar to *S. squalidus*, through F_1 and *S. cambrensis* types, to some individuals resembling *S. vulgaris*. When the F_1 was backcrossed to *S. vulgaris*, progeny were produced which bore a close resemblance to *S. vulgaris*, with some possessing a phenotype very similar to var. *hibernicus*. Detailed analysis of 23 midleaf characters showed that in the F_2, nine characters were intermediate between *S. vulgaris* and *S. squalidus* and 14 were novel, while in the B_1 generation, individuals exhibited a leaf type closer to *S. vulgaris* (Taylor, 1984). Some of the leaf shapes produced in the F_2 were very similar to those typical of York radiate groundsel.

Objectives

The main objective of the work reported in this chapter was to examine the possible route of origin of York radiate groundsel. Results of other studies suggest that three main pathways are most likely to generate hybrid progeny that are tetraploid, partially fertile and similar in morphology to radiate groundsel in the York area; these are via the triploid hybrid *S.* x *baxteri* (Ingram, Weir and Abbott, 1978), via a tetraploid F_1 hybrid formed by the fusion of a normal gamete of *S. vulgaris* and an unreduced gamete of *S. squalidus* (Taylor, 1984) and via a tetraploid F_1 hybrid formed by the fusion of gametes from *S. vulgaris* and tetraploid *S. squalidus* (Taylor, 1984). These three pathways were examined and the morphology of hybrid progeny generated by each pathway were compared to that of established taxa (including York radiate groundsel and *S. vulgaris* var. *hibernicus*).

The outcome of backcrosses between York radiate groundsel and its parental taxa in the field was also examined from crosses made between York radiate groundsel and both *S. vulgaris* and *S. squalidus*. The fertility and morphology of backcross progeny was compared to that of established taxa. The ease with which backcrosses to *S. squalidus* were made was noted as this information can provide insights into the genomic similarity of taxa (Ingram, 1977).

It is possible that York radiate groundsel originated at a location outside York. Thus, a further objective of the studies reported here was to examine the possibility that York radiate groundsel was originally derived from material similar to that in the hybrid swarm at Cork. To this end, a morphometric analysis of the Cork hybrid swarm material, York radiate groundsel and Edinburgh *S. vulgaris* var. *hibernicus* was undertaken.

Finally an attempt was made to establish the current size and distribution of York radiate groundsel populations in the York area. Around York, two other groundsel populations were of interest, one population of non-radiate groundsel (outside the Spotted Cow public house) exhibited a leaf morphology similar to York radiate groundsel, and a small population of *S. vulgaris* var. *hibernicus* grew near Lendal Bridge. To examine the similarity of these two groundsel populations to York radiate groundsel, and to determine the genetic identity of non-radiate and radiate groundsel populations, an isozyme analysis of all mixed populations in York was undertaken.

Methods

Seed collection and plant propagation

All seed generated by experimental crosses or collected from field populations was sown on to damp filter paper. Following germination, seedlings with a root of length 1 cm were transplanted to 11.5 cm pots containing a 1:1 mix of Levingtons M2 compost to gravel. Plants were raised at ambient temperature in a glasshouse under 400 W mercury vapour lamps with the photoperiod set a 16h.

Resynthesis of York radiate groundsel

Crosses

Crosses were made between selected individuals of *S. vulgaris* and *S. squalidus* using the emasculation technique of Ornduff (1964). The terminal 3-4 mm of an unopened capitulum were cut off to remove the anthers. The capitulum was covered with a small bag made from lens tissue and left to mature for 2 to 3 days. Developing stigmas were then examined for presence of pollen and if clean, cross-pollen was dusted onto the stigmas and the capitulum was rebagged until fruit matured. The crossing strategy used to produce hybrid progeny from triploid F_1 plants is outlined in Table 3.3. Five *S.* x *baxteri* plants were synthesized and were easily recognized among offspring raised from a cross. Seed set in the F_1 triploid hybrids was very low but a small amount of open pollinated F_2 seed was collected. Only two of these seed germinated and of the F_2 plants raised, one was totally sterile, while the other exhibited partial fertility. This latter plant was backcrossed to *S. vulgaris* to produce 17 backcross offspring. The same plant also yielded many F_3 progeny through open-pollination (eight of which were analysed further).

One cross between *S. vulgaris* and *S. squalidus* produced a tetraploid F_1 hybrid (YnrxYsq18), presumably from the fusion of a normal gamete of *S. vulgaris* and an unreduced gamete of *S. squalidus*. The crossing strategy used to produce hybrid progeny from this individual is outlined in Table 3.4. The F_1 was partially fertile and 15 F_2 plants were raised for analysis from open-pollinated seed. Backcrosses to *S. vulgaris* were also successful and 11 B_1 plants were raised for analysis.

Crosses were also made between selected individuals of *S. vulgaris* and artificially synthesized tetraploid *S. squalidus*. Tetraploid *S. squalidus* individuals were synthesized by germinating seed in various concentrations of colchicine (0.005% and 0.01% were both effective, although higher concentrations tended to kill seedlings). After germination, seedlings were washed with distilled water and potted up. Tetraploid *S. squalidus* plants exhibited a 'gigas' effect of polyploidization. They were

Table 3.3. Outline of crossing experiments conducted to produce triploid F_1 hybrids between *S. vulgaris* and *S. squalidus* and subsequent backcross and open-pollinated generations (nr=*S. vulgaris* var. *vulgaris*, hib=*S. vulgaris* var. *hibernicus*, sq=*S. squalidus*, York=York radiate groundsel; nrx, indicates a cross where *S. vulgaris* var *vulgaris* is the maternal parent; xnr, where *S. vulgaris* var *vulgaris* is the paternal parent; if no direction is indicated the crosses were reciprocal).

Maternal	*S.vulgaris* var. *vulgaris*	*S.squalidus*	*S. vulgaris* var. *hibernicus*
	x	x	x
Paternal	*S.squalidus*	*S.vulgaris* var. *vulgaris*	*S.squalidus*
crosses made	65	16	80
seeds set	5	13	4
seeds sown	4	10	4
seeds germinated	3	0	2
F_1 obtained	3		2
F_1 plants	**xbax 5**	**xbax 6**	**xbax12**	**xbax 1**	**xbax13**
pollen fertility	32.4	?	?	33.3	32.3
no. pollen pores	2?	3/4	?	3/4	4
mean no florets/capitulum	72.2	80	80.5	67.5	36
Backcrosses	nr sq	nr sq	nr sq	nr sq hib	nr sq hib
crosses made	7 2	4 3	5 1	3 4 4	1 5 1
B_1 seeds set	0 0	0 0	0 0	0 0 0	0 0 0
no. F_1 capitula examined	93	103	59	35	95
seeds set	1	0	2	8	4
seeds sown	1		1	8	4
seeds germinated	1		0	0	1
F_2 analysed	1				1

F_2 plants	**F2EdnrxYsq9**	**F2EdRRxYsq13**
pollen fertility	71.1	male sterile
no. pollen pores	4	
mean no. florets/capitulum	34	60.0
Backcrosses	nrx xnr	nrx xnr
crosses made	2 1	10 8
seeds set	38 50	0 0
seeds sown	38 50	
seeds germinated	8 10	
$B_{1.2}$ obtained	8 10	
$B_{1.2}$ analysed	8 9	
no. F_2 capitula examined	3	17
seeds set	56	0
seeds sown	34	
seeds germinated	13	
F_3 obtained	11	
F_3 analysed	8	

Table 3.4. Outline of crossing experiments to produce tetraploid F_1 hybrids between *S. vulgaris* and *S. squalidus* and subsequent backcross and open-pollinated generations. Table includes cross made between diploid *S. squalidus* and *S. vulgaris* which produced an F_1 tetraploid hybrid (Ynr x Ysq18). Taxon abbreviations; nrx, indicates a cross where *S. vulgaris* var *vulgaris* is the maternal parent; xnr, where *S. vulgaris* var *vulgaris* is the paternal parent.

Maternal	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *vulgaris*	tetraploid *S. squalidus*		
	x	x	x		
Paternal	diploid *S. squalidus*	tetraploid *S. squalidus*	*S. vulgaris* var. *vulgaris*		
crosses made	?	4	26		
seeds set	1	0	8		
seeds sown	1		8		
seeds germinated	1		5		
F_1 obtained					
F_1 analysed	1		4		
F_1 plants	YnrxYsq18	4xSqxYnr6.5.23	4xSq3Ynr1x	4xSq6xYnr1x	4xYs6xYnr25
pollen fertility	67.6	85.3	77.3	83.3	84
no. pollen pores	3/4	3/4	3/4	3/4	3/4
mean no florets/cap.	61	96	82	96	78
Backcrosses	nrx / xnr	nrx / xnr	nrx / xnr	nrx / xnr	nrx / xnr
crosses made	8 / 0	5 / 2	4 / 1	2 / 0	1 / 1
seeds set	213	217 / 46	94 / 29	78	1 / 31
seeds sown	30	25 / 30	40 / 29	30	1 / 31
seeds germinated	17	13 / 15	30 / 29	20	1 / 2
B_1 obtained	14	12 / 14	30 / 26	18	0 / 2
B_1 analysed	11	8 / 8	18 / 19	11	2
no. F_1 caps. examined	10	1	4	2	1
seeds set	65	53	30	45	4
seeds sown	10	10	30	10	4
seeds germinated	24	7	20	8	4
F_2 obtained	18	6	13	6	4
F_2 analysed	15	6	11	4	3

slow growing, with elongated internodes, and produced large leaves, capitula and ligules. Four partially fertile, tetraploid F_1 hybrids were obtained from the crosses outlined in Table 3.4, and a total of 24 F_2 progeny were raised for analysis from open-pollinated seed produced by these plants. F_1 plants were also successfully backcrossed to *S. vulgaris* and 66 B_1 progeny were raised for analysis.

Morphometric comparison

For comparison, 175 plants were grown in a fully randomized design. This included; seven plants of *S. vulgaris* var. *vulgaris*, two plants of var. *hibernicus*, six plants of *S. squalidus*, 11 plants of York radiate groundsel, one tetraploid *S. squalidus* plant, two F_2 progeny of triploid hybrids, eight F_3 progeny of triploid hybrids, 17 backcrosses of F_2 triploid hybrid progeny to *S. vulgaris*, five plants of tetraploid F_1 hybrids, 39 F_2 progeny of tetraploid hybrids and 77 offspring produced after backcrossing tetraploid hybrid progeny to *S. vulgaris*. Sixteen morphometric characters were measured on parental and hybrid progeny. These were; capitulum length (C4), capitulum width (C5), number of outer ray florets (C10), mean outer ray floret length (C11), midleaf length (C14), apical midleaf angle A (C16), midleaf dissection (C18), midleaf standardized perimeter (C19), standardized square of midleaf area (C20), seed length (C21), number of seeds per capitulum (C22), number of Pollen pores (C23), basal auricle width (C35), midleaf width, pollen fertility and proportion open seed set (N.B. labels in parentheses refer to morphological character descriptions in Chapter 2).

For 13 characters (C4, C5, C10, C14, C16, C18, C19, C20, C21, C22, C23, C35 and midleaf width), hybrid and parental taxa were subjected to a principal component analysis (PCA) using the CLUSTAN program (Wishart, 1987). Six groups: *S. vulgaris* var. *vulgaris*, var. *hibernicus*, *S. squalidus*, artificially synthesized tetraploid *S. squalidus*, York radiate groundsel and F1 hybrids between tetraploid *S. squalidus* and *S. vulgaris*, were identified by this procedure. Significant differences in mean phenotype between these six groups was then examined by means of canonical variate analysis (CVA). The 141 F_2, F_3, and B_1 hybrid progeny were then compared to these six groups over all CVA axes and progeny were assigned to a particular group by means of discriminant function analysis (DFA), using the DISCRIM routine of SAS (SAS Institute, Inc., 1990).

Morphometric analysis of hybrids between York radiate groundsel and *S. squalidus*

Reciprocal crosses were made between selected individuals of York radiate groundsel and *S. squalidus* (see Table 3.5). Six F_1 triploid hybrids were generated and another

Table 3.5. Outline of crossing experiments conducted to produce triploid F_1 hybrids between York radiate groundsel and *S. squalidus* and subsequent backcross and open pollinated generations. Taxon abbreviations; nr=*S. vuglaris* var. *vulgaris*, hib=*S. vulgaris* var. *hibernicus*, sq=*S. squalidus*, York=York radiate groundsel; nrx, indicates a cross where *S. vulgaris* var *vulgaris* is the maternal parent; xnr, where *S. vulgaris* var *vulgaris* is the paternal parent, if no direction is indicated the crosses were reciprocal.

Maternal	*S. squalidus*	York radiate groundsel	York radiate groundsel (field)	York radiate groundsel
	x	x	x	x
Paternal	York radiate groundsel	*S. squalidus*	*S. squalidus*	*S. squalidus*
crosses made	13	3	?	?
seeds set	16	1	?	?
seeds sown	2	1	?	?
seeds germinated	2	1	?	?
F_1 obtained	1	1	1	4
F_1 analysed	0	0	0	1
F_1 plants	**SqxYrr2**	**YrrxSq12**	**Field xbax**	**43.42**
pollen fertility	41.9	35.5	?	35.5
no. pollen pores	?	3/4	?	3/4
mean no florets/capitulum	65	79	?	55
Backcrosses	nr, sq, York	nr, York, sq		nr
crosses made	3, 4, 7	2, 8, 8		5
seeds set	0, 0, 0	0, 0, 0		103
seeds sown				40
seeds germinated				27
B_1 analysed				26
no. F_1 capitula examined	137	180	150	5
seeds set	24	62	28	95
seeds sown	9	35	17	15
seeds germinated	9	2	11	7
F_2 obtained	9	2	11	7
F_2 analysed	8	1	0	0
F_2 plants	**F2SqxYrr2**	**F2YrrxSq12**	**F2Fieldxbax**	
pollen fertility	97.1	78.9	?	
no. pollen pores	3	4	?	
mean no. florets/capitulum	23	63	?	
Backcrosses	nrx, nrx	xnr, nrx	xnr	
crosses made	2, 1	2, 1	2	
seeds set	38, 2	4, 2	24	
seeds sown	38, 2	4, 1	24	
seeds germinated	8, 1	1, 1	10	
$B_{1.2}$ obtained	8, 1	0, 0	5	
$B_{1.2}$ analysed	0, 0		5	
no. F_2 capitula examined	26	25	66	
seeds set	154	72	82	
seeds sown	10	10	25	
seeds germinated	3	4	23	
F_3 obtained	2	2	11	
F_3 analysed	1	1	8	

triploid hybrid was obtained naturally from a field experiment. Nine F_2 progeny, were raised from open-pollinated F_1 triploids, while five B_1 progeny were produced when four of the F_1 triploids were backcrossed to *S. vulgaris*. Ten open-pollinated F_3 plants were raised for analysis while backcrossing F_2 plants to *S. vulgaris* produced a further five backcross progeny ($B_{1.2}$).

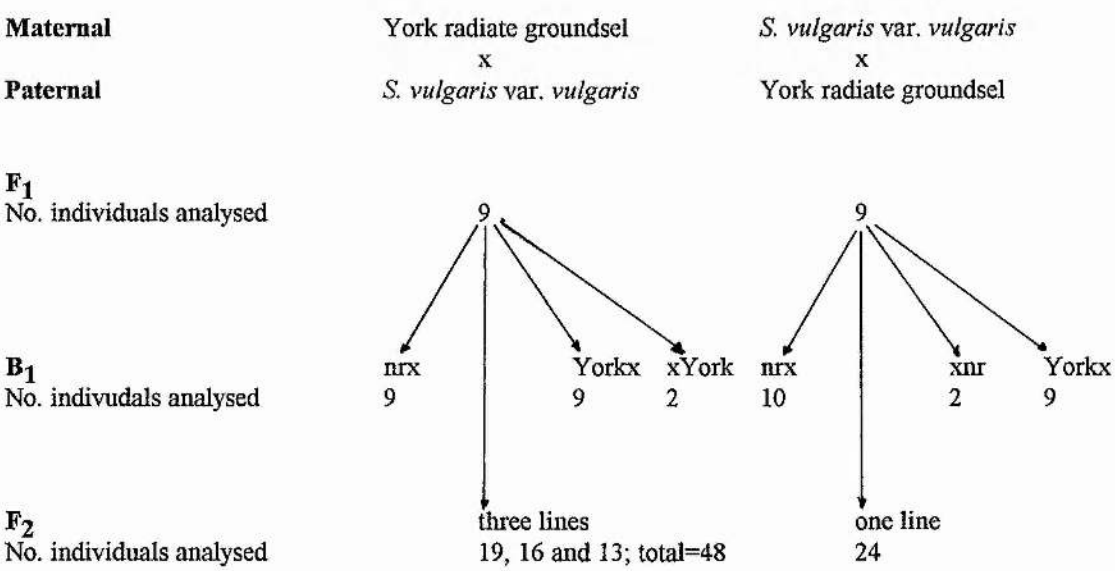
In total, 26 B_1, nine F_2, five $B_{1.2}$ and ten F_3 progeny were raised in a fully randomized design together with the 175 plants grown for morphometric analysis of *S. vulgaris* x *S. squalidus* hybrid progeny (described above). The same 16 morphological characters used to analyse *S. vulgaris* x *S. squalidus* hybrid progeny were measured on each of the 50 York radiate groundsel x *S. squalidus* hybrid progeny examined here. The data set was reduced to 13 characters and combined with the *S. vulgaris* x *S. squalidus* hybrid progeny data set for analysis. PCA, CVA and DFA analytical procedures were conducted as previously described on the entire data set; however, the analysis of York radiate groundsel x *S. squalidus* hybrid progeny will be presented separately in the results section.

One York radiate groundsel x *S. squalidus* F_1 hybrid was chosen for meiotic chromosome analysis. The method followed a protocol described by Crisp (1972) and Ingram (personal communication). Capitula (approximately 2 mm long) containing florets undergoing pollen mother cell meiosis were fixed in freshly prepared Farmer's fluid (1:3, acetic acid:ethanol). A small number of disc florets were dissected out of a capitulum with forceps and placed on a slide in a drop of lacto-propionic orcein for 5-10 minutes. Meiotic tissue was covered with a No. 2 glass cover slip and gently tapped down using the end of a pencil. After staining for half an hour, the cover slip was firmly pressed down onto the slide and the edges sealed with nail varnish. Cells were viewed under oil emersion with a 100 x objective using a normal or phase contrast light microscope. Meiocytes in early development are large granular cells with a refractive callose wall.

Morphometric analysis of hybrids between York radiate groundsel and *S. vulgaris* var. *vulgaris*

Reciprocal crosses were made between selected individuals of York radiate groundsel from Lendal Bridge and *S. vulgaris* var. *vulgaris* from Methil (see Table 3.6). Four F_1 hybrids were initially raised to generate F_2 and B_1 progeny. F_2 seed was collected from capitula on F_1 plants that had been forced to self. A total of 209 individuals were raised from seed in a fully randomized design which included; 33 plants of *S. vulgaris* var. *vulgaris*, five plants of var. *hibernicus*, 40 plants of York radiate

Table 3.6. Outline of crossing experiments between York radiate groundsel and *S. vulgaris* var. *vulgaris* to produce F_1, F_2 and B_1 hybrid progeny for analysis. Taxon abbreviations; nr=*S. vulgaris* var. *vulgaris*, York=York radiate groundsel; nrx, indicates a cross where *S. vulgaris* var *vulgaris* is the maternal parent; xnr, where *S. vulgaris* var *vulgaris* is the paternal parent.



groundsel, 18 F_1 hybrids, 72 F_2 hybrids and 41 reciprocal backcrossed offspring. Plants of York radiate groundsel and both varieties of *S. vulgaris* described here are the same as those raised in the second morphometric study (m2) reported in Chapter 2. The 39 morphological characters used in the second morphometric analysis, were also measured on all hybrid progeny. These included; fourteen characters describing the capitulum, one plant height, 15 midleaf linear measurements, seven midleaf angular measurements, and one the time to apical anthesis.

Morphometric data for all parental taxa and hybrid progeny were analysed by means of a one-way ANOVA to examine differences between means for each character in turn using Tukey-Kramer multiple comparison. Before ANOVA, data were tested for normality and heteroscedasticity and those not conforming were transformed. For 37 characters (excluding measures of fertility) hybrid and parental taxa were subjected to PCA, and component scores were plotted against the first two PCA axes to examine morphological separation. Four groups; *S. vulgaris* var. *vulgaris*, var. *hibernicus*, York radiate groundsel and F_1 hybrids between York radiate groundsel and *S. vulgaris* var. *vulgaris*, were separated in this way and differences in morphology between these classes was assessed by CVA. The 113 F_2 and B_1 hybrid progeny were then compared to these four groups over all CVA axes, and assigned to a particular group by means of discriminant function analysis (DFA), using the DISCRIM routine of SAS (SAS Institute, Inc., 1990)

Morphometric analysis of hybrid swarm material from Cork

Seed was collected from the hybrid swarm at Passage West, Cork in 1991 by R.J. Abbott. From Passage West, ten plants of *S. vulgaris* var. *vulgaris* and 20 plants of hybrid swarm material were analysed together with nine plants of *S. squalidus* from Cork city. Plants were raised from seed, with material grown for the first morphometric study reported in Chapter 2, in a fully randomized design. Plants were taken for first measurement on the day of full anthesis of the apical capitulum. The hybrid swarm material from Passage West, Cork, exhibited a wide range of morphological variation. For ease of analysis, those individuals which possessed a phenotype more similar to York radiate groundsel were classified as Cork radiate groundsel (8 plants) while those which appeared more similar to *S. vulgaris* var. *hibernicus* were classified as such (12 plants). The same 26 morphological characters used in the first morphometric study reported in Chapter 2, were measured on each Cork individual. Fifteen of the characters recorded were descriptors of the capitulum, while nine described vegetative traits, one plant fertility and one time to flowering.

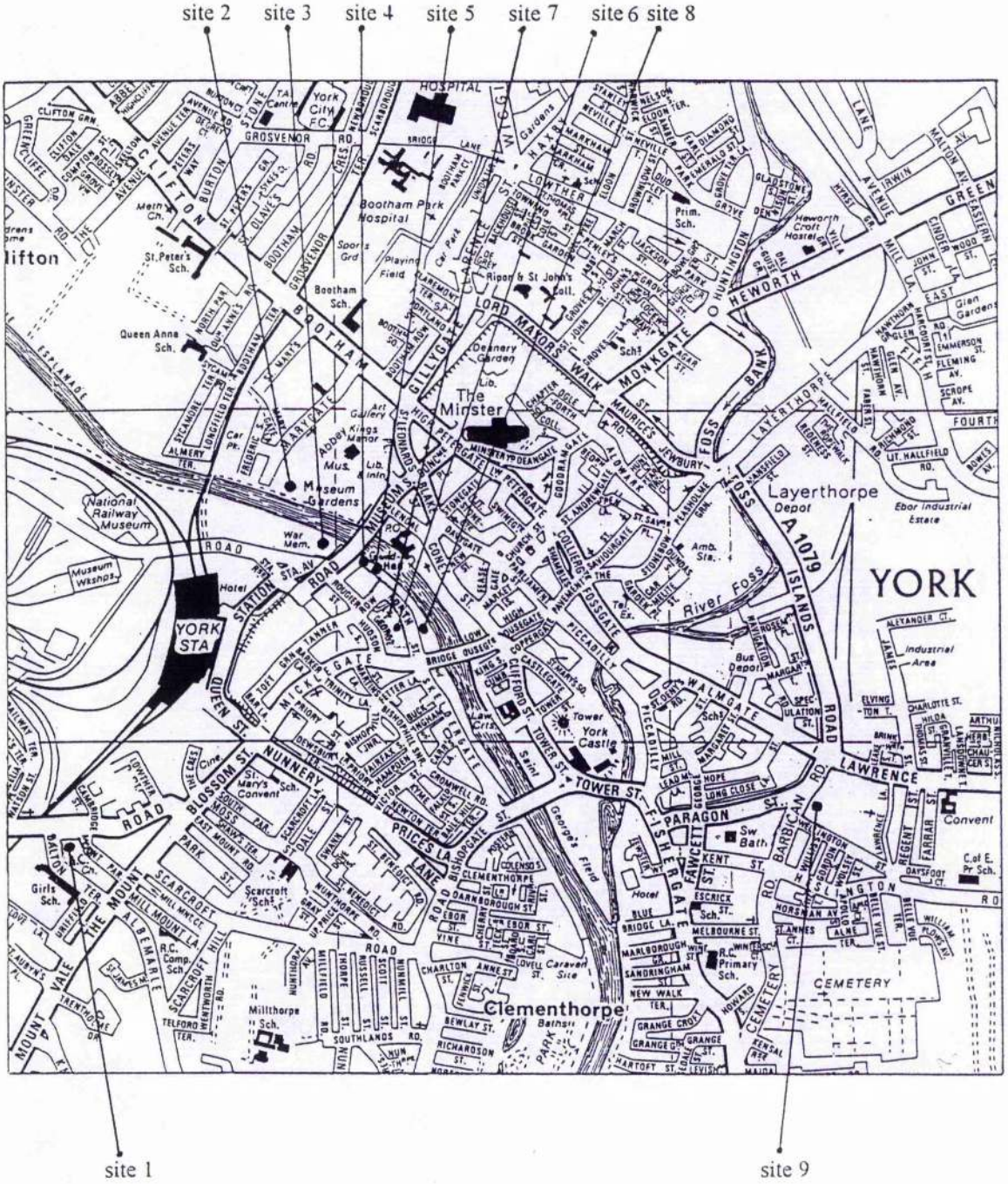


Figure 3.4. Current distribution of radiate groundsel populations in the centre of York; site 1, Dalton Terrace car park; site 2, North West Lendal Bridge; site 3, South West Lendal Bridge; site 4, South East Lendal Bridge; site 5, Lendal Bridge park; site 6, Victoria House car park; site 7, all Saints church; site 8, Viking hotel; site 9, Spotted Cow pub.

The morphometric measurements of ten individuals of York radiate groundsel and 12 individuals of *S. vulgaris* var. *hibernicus*, from the first morphometric study reported in Chapter two, were included in the analysis of Cork material. Each of the 26 morphological characters were examined in turn by means of one-way ANOVA using Tukey-Kramer multiple comparison. Before ANOVA, data were tested for normality and heteroscedasticity and those not conforming were transformed. Morphometric data of the four radiate populations (*S. vulgaris* var. *hibernicus* from Cork and Edinburgh, York radiate groundsel and Cork radiate groundsel) were further subjected to canonical variate analysis (CVA), following the CANDISC procedure of SAS (SAS Institute, Inc., 1990). Characters were used to characterize each radiate population (specified as groups) after verifying that the variables were multivariate normally distributed and assuming equal variances and covariances in each group.

Isozyme analysis of groundsel populations from York

Isozyme variation was surveyed in the progeny of seed collected in 1993 and 1994 from two main populations of *S. vulgaris* var. *vulgaris* and York radiate groundsel growing at Dalton Terrace (site 1, Figure 3.4) and Lendal Bridge in York. The Lendal Bridge population was sub-divided into four sub-populations denoted as, NW (site 2, from which *S. vulgaris* var. *hibernicus* was sampled), SW (site 3), SE (site 4) and Lendal bridge park (site 5). In addition, material was surveyed: from outside the Spotted Cow public house (site 9) which included progeny of 'normal' *S. vulgaris* var. *vulgaris* and also material of *S. vulgaris* var. *vulgaris* which possessed a leaf morphology similar to York radiate groundsel; from Victoria House car park (site 6); and from the Ouse Bridge. Material grown from seed of *S. squalidus* sampled from sites at Dalton Terrace, NW Lendal Bridge, SW Lendal Bridge, All Saints church, Ouse Bridge and outside the spotted cow pub was also subject to analysis.

The following enzyme systems were assayed on each individual; aconitase (ACO), aspartate aminotransferase (AAT), αesterase (αEST), βesterase (βEST), glutamate dehydrogenase (GDH) and Isocitrate dehydrogenase (IDH). Details of electrophoretic and staining procedures are given in Chapter 2. Nei's (1972) genetic distances were calculated from allozyme phenotype frequency data and these measures were used to construct a UPGMA dendrogram using BIOSYS-1 (Swofford and Selander, 1981). It was assumed that tetraploids exhibited disomic inheritance of allozyme variants, and so fixed heterozygous genotypes were coded as single locus diallelic heterozygotes.

Results

Resynthesis of York radiate groundsel

Triploid F_1 hybrids between *S. vulgaris* and *S. squalidus* were quite difficult to generate and crosses were only successful when *S. vulgaris* acted as the maternal parent (Table 3.3). Five triploid hybrids were produced from 145 crosses made with *S. vulgaris* as the maternal parent, while no hybrids were generated from 16 crosses made with *S. squalidus* as the maternal parent. All five triploids were highly sterile (mean pollen stainability 32.67% and mean open seed set 0.0088%). There was no observable difference in fertility between the three triploid hybrids produced using var. *vulgaris* as a parent from the two hybrids produced using var. *hibernicus* as a parent. All attempts to backcross the F_1 hybrids to *S. vulgaris* or *S. squalidus* failed (40 crosses); however, two germinable seed were generated via open-pollination under glass from a total of 385 capitula examined. The two seeds were grown on to produce two F_2 plants One was derived from a cross in which var. *hibernicus* was the maternal parent (EdrrxYsq13) and was totally male sterile producing shrivelled, non-functional anthers. This plant produced no germinable seed when backcrossed to *S. vulgaris* or when left to open pollinate. The other F_2 plant was derived from a cross in which var. *vulgaris* was the maternal parent (EdnrxYsq9), it was partially fertile (pollen stainability = 71.1%; open-seed set = 54.9%), and produced radiate capitula (mean ray floret length = 10.0 mm). No chromosome count was made of this plant which exhibited high open-pollinated seed set and was highly interfertile with *S. vulgaris* (backcross = 86%), and so was presumed to be near tetraploid.

Tetraploid F_1 hybrids were relatively easy to generate, although crosses were only successful when tetraploid *S. squalidus* acted as the maternal parent (Table 3.4). Four tetraploid hybrids were produced from 26 crosses made with tetraploid *S. squalidus* as the maternal parent, while no hybrid progeny were produced from four crosses made when *S. vulgaris* acted as the maternal parent. In addition, one 'spontaneous' tetraploid F_1 hybrid (YnrxYsq18) was produced by crossing diploid *S. squalidus* to tetraploid *S. vulgaris* (maternal parent) and was similar in overall morphology and fertility to the tetraploid hybrids generated using tetraploid *S. squalidus*. All five tetraploid F_1 hybrids were partially fertile (mean pollen stainability = 79.5%; mean open-seed set = 20.7%) and when left to open pollinate, copious amounts of F_2 seed were collected from each plant. All tetraploid F_1 hybrids were also partially interfertile with *S. vulgaris* and mean seed set of these backcrosses was 33.9%.

Means and standard deviations for 16 morphological and fertility characters measured on all parental material and hybrid progeny are presented in Table 3.7. The first two

Table 3.7. Means and standard deviations (in parentheses) for 16 morphologcial and fertility characters measured on all triploid and tetraploid F_1 hybrids between *S. vulgaris* and *S. squalidus* and their open pollinated and backcrossed (from crosses outlined in tables 3.5 and 3.6). Values for parental taxa raised at the same time are included. Values for hybrid progeny derived from crosses between York radiate groundsel and *S. squalidus* are included in this table for comparison.

Taxon/cross	N=	C4 Capitulum length	C5 Capitulum width[a]	C10 Number of ray florets	C11 Mean ray floret len.[a]	C21 Seed length	C22 No. of seeds per capitulum[a]	C23 Number of pollen pores	% Pollen stainability	% Open seed set
S. vulgaris v *vul.*	7	0.99 (0.11)	0.37 (0.04)	0.0 (0)	0.05 (0)	0.236 (0.05)	68.0 (31.2)	3.04 (0.09)	83.5 (17.4)	82.5 (33.7)
S. squalidus	6	0.95 (0.05)	0.57 (0.04)	12.2 (0.9)	1.11 (0.24)	0.228 (0.02)	74.7 (11.2)	3.00 (0)	94.5 (9.6)	72.3 (31.1)
S. vulgaris v *hib*	2	0.77 (0.11)	0.30 (0)	8.5 (0.7)	0.50 (0)	0.248 (0.00)	47.0 (7.1)	3.12 (0.18)	71.0 (33.0)	89.2 (1.6)
York radiate	11	0.88 (0.03)	0.44 (0.08)	8.6 (0.8)	0.44 (0.08)	0.293 (0.01)	57.8 (10.6)	3.86 (0.21)	77.5 (21.2)	74.3 (24.9)
tet. *S. squalidus*	1	0.80	0.75	13.0	1.20	0.282	83.0	3.00	98.2	50.0
F_1 4xsq x nr	4	0.94 (0.07)	0.72 (0.05)	12.7 (0.5)	1.01 (0.06)	0.303 (0.01)	85.3 (9.4)	3.00 (0)	82.5 (3.6)	38.6 (6.3)
F_2 4xsq x nr	24	0.99 (0.07)	0.46 (0.06)	12.0 (1.9)	0.73 (0.28)	0.274 (0.04)	66.4 (21.7)	3.19 (0.19)	77.1 (14.4)	27.9 (27.3)
B_1 4xsq x nr	66	0.97 (0.08)	0.45 (0.07)	9.8 (3.4)	0.25 (0.11)	0.316 (0.47)	62.4 (14.9)	3.25 (0.30)	68.8 (13.5)	28.8 (18.4)
F_1 YnrxYsq18	1	1.00	0.70	13.0	1.00	0.298	61.0	3.00	67.7	39.3
F_2 YnrxYsq18	15	1.02 (0.09)	0.46 (0.03)	10.0 (1.8)	0.83 (0.24)	0.281 (0.03)	61.1 (9.1)	3.18 (0.27)	83.9 (11.4)	27.0 (14.2)
B_1 YnrxYsq18	11	1.01 (0.05)	0.45 (0.05)	9.8 (1.9)	0.26 (0.09)	0.297 (0.03)	69.7 (31.8)	3.32 (0.34)	70.3 (10.0)	24.7 (15.1)
F_2 EdnrxYSq9	1	0.90	0.50	10.0	1.10	0.298	34.0	4.00	71.2	32.4
F_3 EdnrxYSq9	8	1.09 (0.07)	0.48 (0.06)	10.6 (1.8)	1.14 (0.09)	0.290 (0.03)	54.8 (10.8)	3.41 (0.26)	65.9 (15.8)	16.3 (20.9)
$B_{1.2}$ EdnrxYsq9	17	0.96 (0.09)	0.43 (0.05)	11.0 (2.1)	0.45 (0.19)	0.275 (0.03)	57.3 (11.1)	3.37 (0.38)	60.8 (15.5)	29.2 (13.5)
F_2 EdnrxYsq13	1	0.60	0.50	13.0	1.10	sterile	60.0	3.00	male sterile	0.0
Progeny of hybrids derived from crosses between York radaite groundsel and *S.squalidus*										
F_1 43.42	1	0.80	0.50	10.0	1.00	0.294	82.0	3.00	97.1	25.0
F_2 Ynrx Sq12	1	0.90	0.70	10.0	1.00	0.286	84.0	3.50	35.5	23.0
F_2 SqxYnr2	8	0.85 (0.09)	0.65 (0.14)	11.7 (2.1)	1.06 (0.24)	0.296 (0.63)	90.0 (13.2)	3.22 (0.36)	73.8 (35.6)	24.0 (10.4)
B_1 43.42	26	1.01 (0.12)	0.47 (0.09)	10.7 (2.1)	0.57 (0.32)	0.275 (0.54)	65.3 (17.1)	3.32 (0.33)	65.5 (15.9)	22.8 (13.6)
F_3 Field xbax & F_3 YnrxSq12 & F_3 SqxYnr2	10	0.91 (0.12)	0.47 (0.06)	12.1 (1.7)	0.89 (0.28)	0.246 (0.04)	68.3 (13.4)	3.16 (0.18)	55.4 (35.1)	9.7 (14.4)
$B_{1.2}$ Field xbax	5	1.00 (0.10)	0.45 (0.04)	11.2 (1.6)	0.62 (0.13)	0.301 (0.04)	58.2 (16.4)	3.15 (0.22)	69.1 (9.2)	13.3 (9.8)

Table 3.7. Continued.

Taxon/cross	N=	C14 Midleaf length[a]	Midleaf width	C16 Apical angle	C35 Basal auricle width[a]	C19 Leaf perimeter[b]	C20 Square of leaf area[b]	C18 Midleaf dissection[c]
S. vulgaris v *vul.*	7	10.7 (4.2)	3.81 (1.0)	22.9 (10.9)	1.29 (0.41)	4.73 (1.26)	1.30 (0.42)	13.0 (0.9)
S. squalidus	6	10.0 (0.9)	3.53 (0.7)	96.0 (5.3)	0.76 (0.36)	4.17 (1.74)	1.03 (0.19)	12.7 (3.9)
S. vulgaris v *hib*	2	8.2 (0.7)	3.25 (0.3)	131.5 (4.9)	0.63 (0.18)	3.92 (0.33)	1.28 (0.02)	9.9 (0.3)
York radiate	11	12.3 (2.7)	5.60 (1.4)	95.5 (16.3)	1.82 (0.95)	6.64 (1.24)	1.67 (0.45)	17.9 (2.4)
tet. *S. squalidus*	1	18.6	8.90	115.0	0.50	5.59	2.09	16.7
F_1 4xsq x nr	4	8.7 (0.5)	4.87 (1.6)	100.2 (15.7)	0.70 (0.61)	5.80 (5.80)	1.26 (0.42)	14.8 (5.1)
F_2 4xsq x nr	24	6.5 (1.5)	3.14 (1.1)	108.3 (19.4)	0.75 (0.27)	4.05 (1.16)	0.88 (0.24)	11.1 (2.7)
B_1 4xsq x nr	66	8.0 (1.6)	3.92 (1.2)	119.5 (12.7)	0.97 (0.54)	4.90 (1.21)	1.22 (0.35)	12.8 (2.8)
F_1 YnrxYsq18	1	8.6	5.40	111.0	0.60	7.67	1.28	19.9
F_2 YnrxYsq18	15	8.1 (2.1)	3.92 (0.7)	116.7 (17.7)	1.03 (0.29)	5.38 (1.21)	1.09 (0.21)	14.5 (2.9)
B_1 YnrxYsq18	11	9.9 (1.0)	5.30 (1.0)	111.9 (20.9)	1.87 (0.70)	6.11 (1.48)	1.53 (0.36)	15.7 (3.2)
F_2 EdnrxYSq9	1	5.7	4.00	90.0	0.50	6.31	0.88	16.1
F_3 EdnrxYSq9	8	9.2 (1.3)	4.59 (0.9)	06.6 (18.6)	0.81 (0.28)	5.81 (0.61)	1.27 (0.29)	15.7 (1.3)
$B_{1.2}$ EdnrxYsq9	17	8.3 (2.1)	3.65 (0.8)	116.1 (11.4)	0.79 (0.29)	4.84 (1.06)	1.17 (0.44)	12.2 (2.2)
F_2 EdnrxYsq13	1	13.8	0.80	64.0	0.40	0.51	0.36	3.1
Progeny of hybrids derived from crosses between York radaite groundsel and *S.squalidus*								
F_1 43.42	1	10.6	5.20	80.0	0.90	6.88	1.69	17.2
F_2 Ynrx Sq12	1	16.2	8.10	141.0	1.00	6.85	2.78	16.5
F_2 SqxYn2	8	9.1 (2.2)	5.27 (1.4)	117.7 (13.1)	0.69 (0.43)	7.06 (2.46)	1.37 (0.33)	18.0 (5.6)
B_1 43.42	26	8.8 (1.9)	4.01 (0.9)	113.5 (22.6)	0.70 (0.31)	4.63 (1.21)	1.21 (0.35)	12.5 (2.9)
F_3 Field xbax & F_3 YnrxSq12 & F_3 SqxYn2	10	7.1 (1.4)	3.09 (1.6)	88.1 (26.3)	0.71 (0.51)	4.52 (2.26)	0.82 (0.39)	13.2 (5.3)
$B_{1.2}$ Field xbax	5	7.3 (2.1)	3.58 (1.2)	20.2 (9.8)	0.76 (0.29)	5.39 (0.97)	1.22 (0.35)	13.2 (3.1)

[a] Log^e transformed

[b] Calculated as midleaf perimeter divided by the square root of area; high ratio indicating highly divided leaf

[c] Perimeter and square of area measures were divided by midleaf length to standardize.

components of a PCA on 13 morphological characters clearly differentiated individuals from the following six groups: *S. squalidus*, tetraploid *S. squalidus*, *S. vulgaris* var. *vulgaris* and var. *hibernicus*, York radiate groundsel and tetraploid F_1 hybrids between *S. squalidus* and *S. vulgaris*. Examination of Mahalonobis' distances produced by canonical variate analysis revealed that the differences in mean phenotype between these groups were significant ($P < 0.001$). Following comparison of all CVA axes, DFA assigned all 141 hybrid progeny, produced by all resynthesis pathways, into one of these six groups at the 99.9% significance level (Table 3.8). The first two PCA axes were plotted against each other to demonstrate the morphological differentiation of parental taxa and hybrid progeny; for ease of presentation three separate plots are illustrated (Figures 3.5, 3.6 and 3.7)

In Figure 3.5, plots of scores for the first two principal components are illustrated for parental taxa and hybrid progeny obtained from triploid F_1 hybrids, together with the progeny of the partially fertile F_2 hybrid (EdnrxYsq9). The first component separated York radiate groundsel from *S. squalidus* and *S. vulgaris*, while the second component separated *S. squalidus*, *S. vulgaris* var. *vulgaris* and var. *hibernicus* groups from each other. PCA placed the F_2 hybrid, EdnrxYsq9, very close to *S. vulgaris* var. *hibernicus* indicating their close morphological similarity, while the male sterile F_2 hybrid, EdrrxYsq13, which exhibited long thin leaves and long internodes, is placed close to *S. squalidus*. The B_1 and F_3 progeny of EdnrxYsq9 showed considerable morphological variation, and DFA (Table 3.8) assigned three F_3 and four B_1 plants to the York radiate groundsel grouping and seven B_1 plants to the *S. vulgaris* var. *hibernicus* group. Other parental classes were also represented in the hybrid progeny apart from var. *vulgaris*.

In Figure 3.6, results of the PCA are illustrated for parental taxa and hybrid progeny of crosses between tetraploid *S. squalidus* and *S. vulgaris* (4xSqxnr). The first and second components placed tetraploid F_1 hybrids in an intermediate position between tetraploid *S. squalidus* and *S. vulgaris*. The B_1 and F_2 progeny of the 'synthesized' tetraploid F_1 hybrid showed considerable morphological variation, and DFA assigned three F_2 and 10 B_1 progeny to the York radiate groundsel grouping and seven F_2 and 25 B_1 individuals to the *S. vulgaris* var. *hibernicus* group (Table 3.8). Other hybrid progeny were assigned by DFA into either the *S. squalidus*, tetraploid *S. squalidus* or tetraploid F_1 hybrid groupings, while four B_1 progeny were classified as *S. vulgaris* var. *vulgaris*. A few more *S. vulgaris* var. *hibernicus*- and York radiate groundsel-like hybrids were produced from 'synthesized' tetraploid F_1 hybrids that were

Table 3.8. Listing of discriminant function morphometric classification of 141 hybrid progeny produced from crosses between *S. vulgaris* and *S. squalidus*, based on all canonical variate axes, into one of six parental classes significantly differentiated by CVA.

DFA parental classes Hybrid progeny classes	*S. vulgaris* var. *vulgaris*	*S. vuglaris* var. *hibernicus*	York radiate groundsel	*S. squalidus*	Tetraploid *S. squalidus*	Tetraploid F_1 hybrids	Total
F_2 4xsq x nr		7	3	5	6	3	24
B_1 4xsq x nr	4	25	10	7	5	15	66
F_2 YnrxYsq18		4		5	1	5	15
B_1 YnrxYsq18		3	3	2		3	11
F_3 EdnrxYsq9			3			5	8
$B_{1.2}$ EdnrxYsq9		7	4	1	2	3	17
Total	4	46	23	20	14	34	141

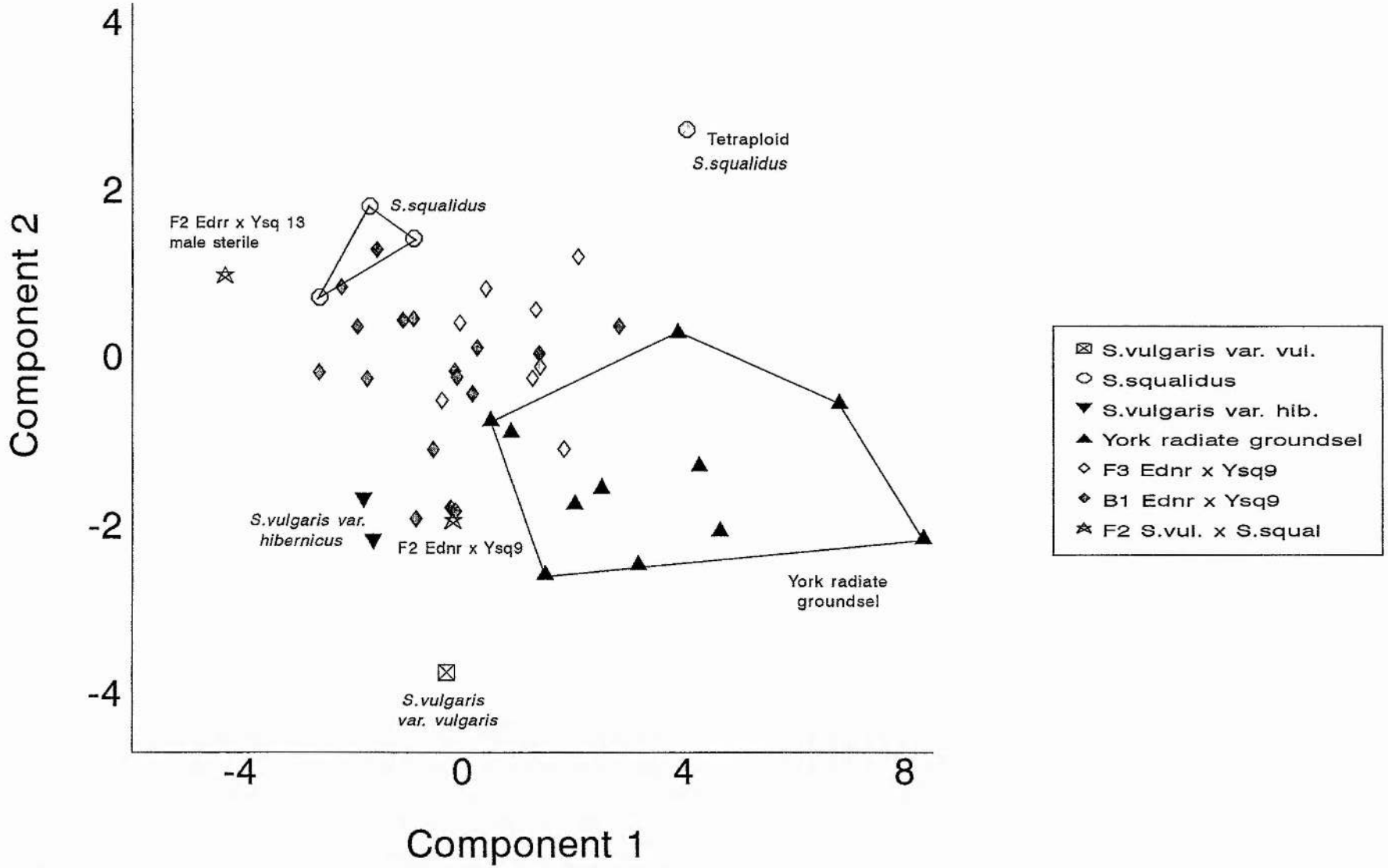


Figure 3.5. Plot of the scores (component 1 vs component 2), following principal component analysis of 13 morphological characters, for parental taxa and hybrid progeny derived from triploid F_1 hybrids generated after crossing *S. squalidus* and *S. vulgaris*. The first three eigen values were 4.01, 1.89 and 1.44 and described 30.8, 14.5 and 11.1% of the variance in the data respectively. Characters that contributed most heavily to the first principal component axis (with values of the eigenvectors shown in parentheses) were: C14, Midleaf length (0.331); Midleaf width (0.465); C18, Midleaf dissection (0.401); C19, Midleaf standardised perimeter (0.417); C20, Midleaf standardised area (0.402); and C35, Midleaf basal auricle width (0.319). Characters contributing most to the second principal component axis were; C10, Number of ray florets (0.405); C23, Pollen pore number (-0.213); C5, Capitulum width (0.534); C14, Midleaf length (0.223); C22, Number of seeds (0.412); and C21, Seed length (0.220).

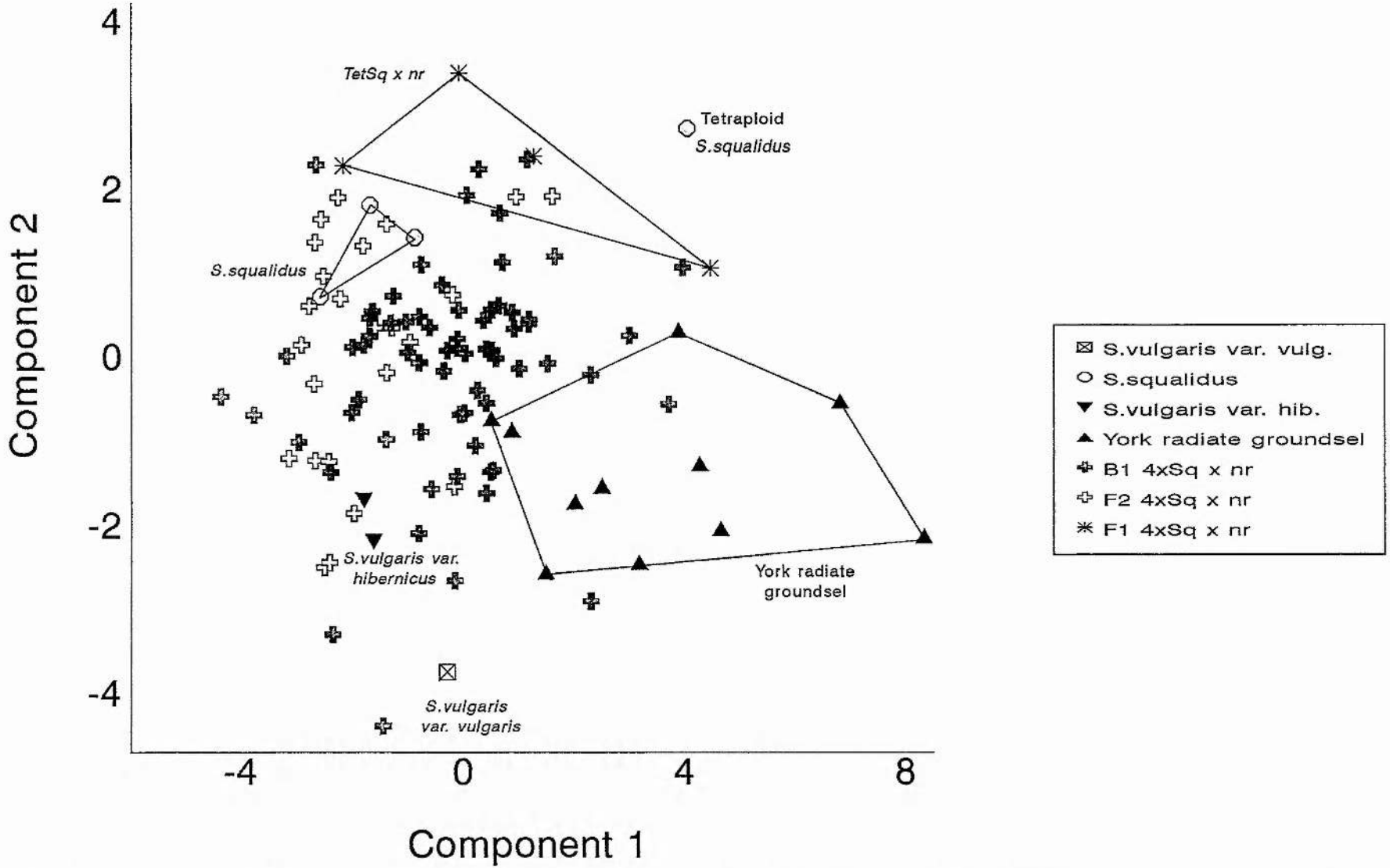


Figure 3.6. Plot of the scores (component 1 vs component 2), following principal component analysis of 13 morphological characters, for parental taxa and hybrid progeny produced from crosses between tetraploid *S. squalidus* and *S. vulgaris*. The first three eigen values were 4.01, 1.89 and 1.44 and described 30.8, 14.5 and 11.1% of the variance in the data respectively. Characters that contributed most heavily to the first principal component axis (with values of the eigenvectors shown in parentheses) were: C14, Midleaf length (0.331); Midleaf width (0.465); C18, Midleaf dissection (0.401); C19, Midleaf standardised perimeter (0.417); C20, Midleaf standardised area (0.402); and C35, Midleaf basal auricle width (0.319). Characters contributing most to the second principal component axis were; C10, Number of ray florets (0.405); C23, Pollen pore number (-0.213); C5, Capitulum width (0.534); C14, Midleaf length (0.223); C22, Number of seeds (0.412); and C21, Seed length (0.220).

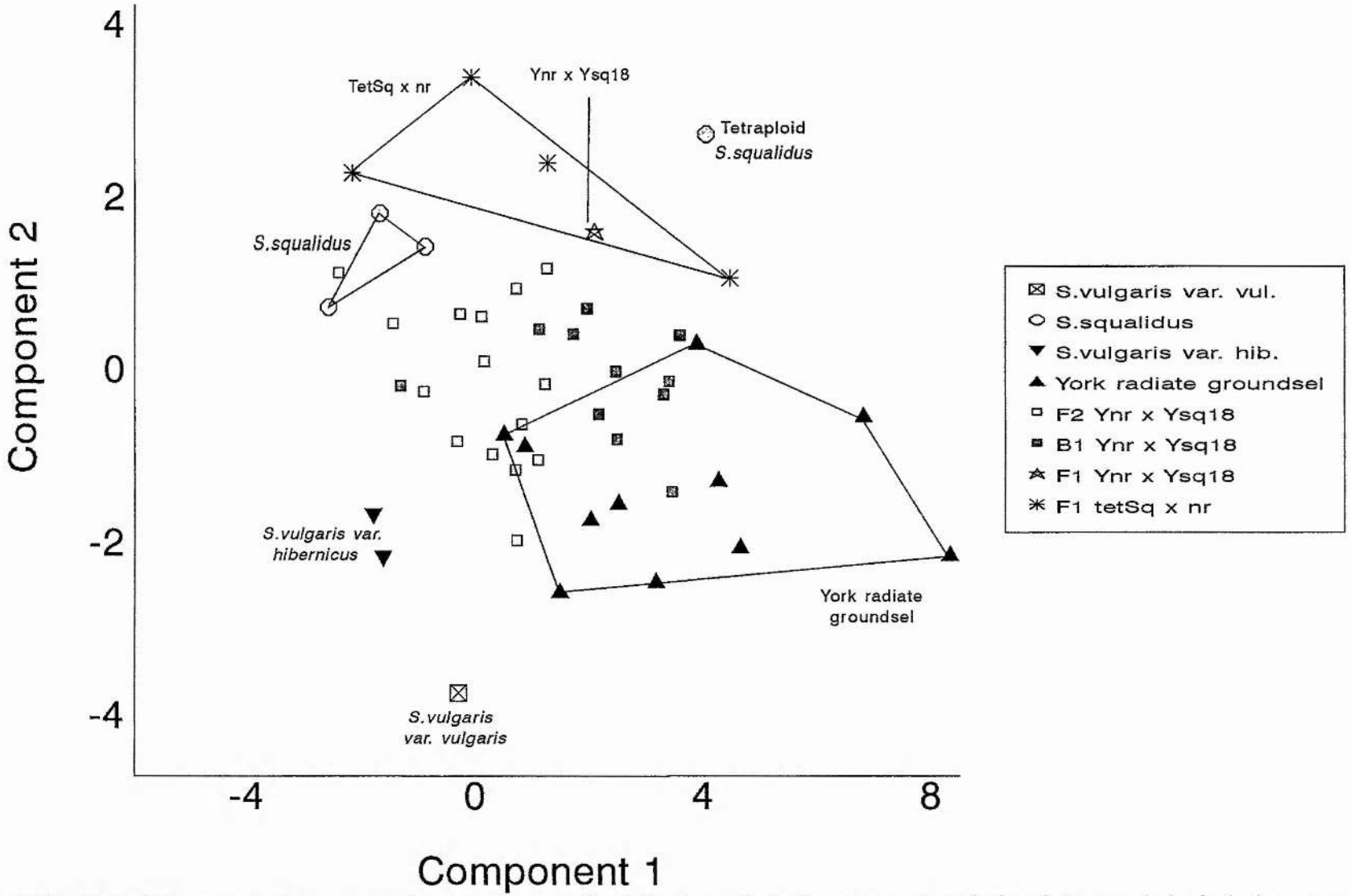


Figure 3.7. Plot of the scores (component 1 vs component 2), following principal component analysis of 13 morphological characters, for parental taxa and hybrid progeny produced from a 'spontaneous' tetraploid F_1 hybrid generated after crossing diploid *S. squalidus* and *S. vulgaris*. The first three eigen values were 4.01, 1.89 and 1.44 and described 30.8, 14.5 and 11.1% of the variance in the data respectively. Characters that contributed most heavily to the first principal component axis (with values of the eigenvectors shown in parentheses) were: C14, Midleaf length (0.331); Midleaf width (0.465); C18, Midleaf dissection (0.401); C19, Midleaf standardised perimeter (0.417); C20, Midleaf standardised area (0.402); and C35, Midleaf basal auricle width (0.319). Characters contributing most to the second principal component axis were; C10, Number of ray florets (0.405); C23, Pollen pore number (-0.213); C5, Capitulum width (0.534); C14, Midleaf length (0.223); C22, Number of seeds (0.412); and C21, Seed length (0.220).

backcrossed to *S. vulgaris* (53%) than were generated in the F_2 (41.6%). Plants resembling var. *vulgaris* were only produced in the B_1 offspring class.

Finally, in Figure 3.7, the first two principal component scores are plotted of parental taxa and hybrid progeny of the 'spontaneous' tetraploid F_1 hybrid (YnrxYsq18) produced by crossing *S. vulgaris* with diploid *S. squalidus*, together with the F_1 progeny of crosses between tetraploid *S. squalidus* and *S. vulgaris*. In this plot the 'spontaneous' tetraploid F_1 hybrid is associated with the 'synthesized' tetraploid F_1 hybrids. F_2 and B_1 progeny showed considerable morphological variation, and DFA assigned four F_2 and three B_1 plants into the *S. vulgaris* var. *hibernicus* grouping and three B_1 individuals into the York radiate groundsel group (Table 3.8). Other parental classes were also represented in the hybrid progeny apart from var. *vulgaris*.
Taken overall, DFA showed that 49% of 141 hybrid progeny generated from crosses were similar in morphology to York radiate groundsel or *S. vulgaris* var. *hibernicus*, while 24% were similar to tetraploid F_1 hybrids.

Morphometric analysis of crosses between York radiate groundsel and *S. squalidus*

Six triploid F_1 hybrids were generated from crosses between York radiate groundsel and *S. squalidus*. Five of these were produced using York radiate groundsel as the maternal parent (43.42 and YrrxSq12), while one was formed using *S. squalidus* as the maternal parent (SqxYrr2). It was notable that triploid hybrids were easier to synthesize between York radiate groundsel and *S. squalidus* (two hybrids were generated from 16 crosses) than between *S. vulgaris* and *S. squalidus* (see above section). A triploid F_1 hybrid that was generated spontaneously in a field experiment (Field xbax) was also included in analysis. On average, triploid hybrids exhibited a pollen fertility of 36.6% and open seed set of 0.63%, and some germinable seed was collected from all triploids. Some York radiate groundsel x *S. squalidus* hybrids were backcrossed to *S. vulgaris* and exhibited high fertility (mean seed set of backcrosses = 37.4%); however, other backcrosses failed. Twenty two F_2 progeny were grown on , of which nine were analysed. These exhibited a mean pollen stainability of 88% and open seed set of 5.6%. Nonetheless, numerous open pollinated F_3 seed were collected from all F_2 plants. Also, some successful backcrosses to *S. vulgaris* were generated ($B_{1.2}$).

Means and standard deviations of 16 morphological and fertility characters measured on parental material and hybrid progeny are presented in Table 3.7. As for *S. vulgaris* x *S. squalidus* hybrids, PCA analysis of 13 morphological characters differentiated

Table 3.9. Listing of discriminant function morphometric classification of 50 hybrid progeny produced from crosses between York radiate groundsel and *S. squalidus*, based on all canonical variate axes, into one of six parental classes significantly differentiated by CVA.

Discriminant classes	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *hibernicus*	York radiate groundsel	*S. squalidus*	Tetraploid *S. squalidus*	Tetraploid F_1 hybrids	Total
Hybrid progeny							
F_2 YrrxSq12						1	1
F_2 SqxYrr2				1	2	5	8
B_1 43.42		6	9	1		10	26
F_3 field xbax & F_3 YrrxSq12 & F_3 SqxYrr2		2	2	4	1	1	10
$B_{1.2}$ Field xbax		2	1	1		1	5
Total	0	10	12	7	3	18	50

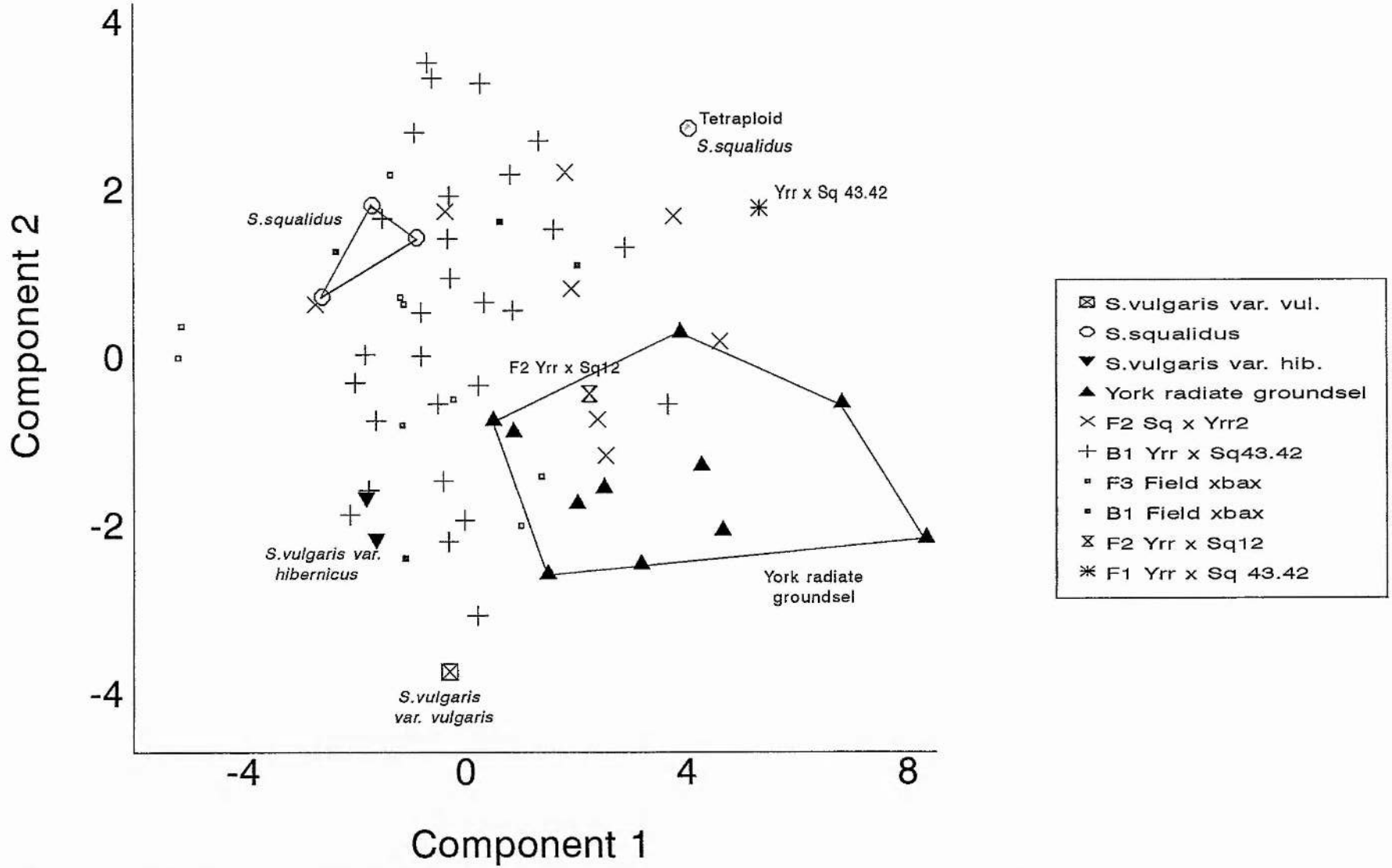


Figure 3.8. Plot of the scores (component 1 vs component 2), following principal component analysis of 13 morphological characters, for parental taxa and hybrid progeny derived from triploid F_1 hybrids generated after crossing *S. squalidus* and York radiate groundsel. The first three eigen values were 4.01, 1.89 and 1.44 and described 30.8, 14.5 and 11.1% of the variance in the data respectively. Characters that contributed most heavily to the first principal component axis (with values of the eigenvectors shown in parentheses) were: C14, Midleaf length (0.331); Midleaf width (0.465); C18, Midleaf dissection (0.401); C19, Midleaf standardised perimeter (0.417); C20, Midleaf standardised area (0.402); and C35, Midleaf basal auricle width (0.319). Characters contributing most to the second principal component axis were; C10, Number of ray florets (0.405); C23, Pollen pore number (-0.213); C5, Capitulum width (0.534); C14, Midleaf length (0.223); C22, Number of seeds (0.412); and C21, Seed length (0.220).

individuals from the following six groups; *S. squalidus*, tetraploid *S. squalidus*, *S. vulgaris* var. *vulgaris* and var. *hibernicus*, York radiate groundsel and tetraploid F_1 hybrids between *S. squalidus* and *S. vulgaris*, and CVA showed that the differences in mean phenotype were significant ($P < 0.001$). Following comparison of all CVA axes, DFA assigned all 50 hybrid progeny into one of these six groups at the 99.9% significance level (Table 3.9). The first two components of the PCA are plotted against each other to demonstrate morphological differentiation of parental taxa and hybrid progeny (Figure 3.8). The first and second components placed the triploid F_1 hybrid, 43.42, close to tetraploid *S. squalidus*. The B_1, F_2 and F_3 hybrid progeny showed considerable morphological variation. DFA (Table 3.9) assigned 12 of these progeny to the York radiate groundsel grouping, and 10 to the *S. vulgaris* var. *hibernicus* group, which together make up 44% of the total hybrid progeny analysed. Other parental classes were represented in the hybrid progeny apart from var. *vulgaris*. An examination of meiotic cells in one York radiate groundsel x *S. squalidus* hybrid revealed that on average, two trivalents, and between four and eight univalents were produced, with the remaining chromosomes forming bivalents.

Morphometric analysis of crosses between York radiate groundsel and *S. vulgaris* var. *vulgaris*

F_1 hybrids between York radiate groundsel and *S. vulgaris* were easy to generate and 136 seed were produced from 32 crosses. Four F_1 progeny were raised to maturity and produced large numbers of F_2 seed under conditions of selfing. These F_1 plants were also highly interfertile with both parent taxa and reciprocal B_1 generations were produced.

Means and standard deviations of the 41 morphological and life history characters measured on parent material and hybrid progeny are presented in Table 3.10. One-way ANOVA of each character in turn, revealed that *S. vulgaris* var. *vulgaris* and York radiate groundsel had significantly different means for 25 of the characters. For 12 of these characters, F_2 progeny exhibited a range of values between parental extremes, although mean phenotype tended to be intermediate between parent means, and the B_1 progeny were more similar in morphology to the taxon to which they had been backcrossed (C4, C6, C8, C10, C11, C15, C21, C23, C28, C29, C34 and C46). For two characters, F_1 and F_2 progeny were more similar in mean phenotype to York radiate groundsel (C3 and C4), while for four characters such hybrid progeny were more similar in mean phenotype to *S. vulgaris* var. *vulgaris* (C2, C9, C38 and C44). For seven characters, all hybrid progeny (F_1, F_2 and B_1) exhibited a mean phenotype intermediate between that exhibited by York radiate groundsel and *S. vulgaris* var.

Table 3.10. Means, standard deviations, significant differences (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, not significant) and results of Tukey-Kramer multiple comparison for 41 morphological traits measured York radiate groundsel and *S. vulgaris* var. *vulgaris* parent material and F_1, F_2 and reciprocal backcr generations, and five individuals of *S. vulgaris* var. *hibernicus*. For F_2 progeny the original maternal paren the F_1 cross is listed, while for B_1 progeny the taxon to which F_1 progeny were backcrossed is also list Means sharing the same superscript are not significantly different ($P \leq 0.05$) and standard deviations shown in parentheses.

Taxa	*S. vulgaris* v. *vulgaris*	*S. vulgaris* v. *hibernicus*	York radiate groundsel	F_1	F_2 York radiate groundsel	F_2 *S. vulgaris* v. *vulgaris*	B_1 *S. vulgaris* v. *vulgaris*	B_1 York radiate groundsel	
N=	33	5	40	18	48	24	21	20	
Character									*P*
C1 Plant height (cm)	22.1^{ab} (7.1)	23.5^{ab}(4.9)	21.5^{b} (3.1)	21.9^{ab}(2.5)	22.3^{ab}(5.7)	26.5^{a} (6.3)	24.7^{ab}(7.4)	21.9^{ab}(5.2)	•
C2 Infloresence length[f] (cm)	1.17^{b}(0.25)	1.85^{a}(0.66)	1.78^{a}(0.47)	1.60^{a}(0.36)	1.59^{a}(0.51)	1.72^{a}(0.39)	1.59^{a}(0.38)	1.72^{a}(0.56)	***
C3 Pedicel length[f] (cm)	0.45^{b}(0.26)	1.09^{a}(0.65)	0.97^{a}(0.46)	0.82^{a}(0.33)	0.85^{a}(0.49)	0.93^{a}(0.37)	0.79^{a}(0.45)	0.86^{a}(0.53)	***
C4 Capitulum length (cm)	0.73^{c}(0.09)	0.86^{abc}(0.03)	0.81^{ab}(0.06)	0.78^{bc}(0.06)	0.77^{bc}(0.08)	0.79^{ab}(0.07)	0.80^{ab}(0.08)	0.86^{a}(0.11)	***
C6 Number of phyllaries	19.7^{a} (2.0)	20.8^{ab}(1.3)	15.3^{d} (1.6)	18.6^{ab}(2.6)	18.9^{ab}(2.1)	17.5^{bc}(2.9)	20.2^{a} (1.9)	16.2^{cd}(2.7)	***
C8 Number of calyculous bracts	10.4^{b} (1.8)	13.0^{ab}(2.6)	5.3^{d} (1.3)	8.1^{c} (1.8)	9.7^{bc}(2.6)	8.8^{bc}(2.4)	11.1^{b} (2.1)	7.5^{c} (2.2)	***
C9 Mean calyculous bract length (cm)	0.29^{b}(0.13)	0.29^{b}(0.02)	0.39^{a}(0.03)	0.28^{b}(0.03)	0.30^{b}(0.04)	0.27^{b}(0.04)	0.28^{b}(0.04)	0.32^{b}(0.04)	***
C10 Number of ray florets	0.0^{c} (0)	8.0^{a} (2.8)	7.9^{a} (0.9)	8.5^{a} (0.8)	4.0^{b} (4.5)	6.2^{ab}(3.8)	0.7^{c} (2.4)	6.0^{ab}(4.1)	***
C11 Mean outer floret length[f] (cm)	0.19^{d}(0.01)	0.39^{ab}(0.02)	0.42^{a}(0.05)	0.25^{c}(0.03)	0.26^{c}(0.09)	0.30^{bc}(0.1)	0.22^{b}(0.02)	0.36^{cd}(0.12)	***
C15 Number of midleaf lobes	10.7^{d} (1.0)	11.4^{cd}(1.1)	15.9^{a} (1.7)	12.6^{c} (1.0)	12.9^{c} (1.8)	13.7^{bc}(1.8)	10.9^{d} (1.6)	13.9^{b} (2.8)	***
C18 Leaf dissection[g]	13.1^{c} (1.9)	11.0^{c} (1.1)	18.9^{a} (2.9)	16.4^{ab}(4.5)	15.8^{b} (3.3)	15.5^{bc}(4.1)	14.0^{bc}(3.3)	14.6^{bc}(3.9)	***
C19 Standardized leaf perimeter[h]	4.79^{b}(0.99)	3.98^{b}(0.38)	6.00^{a}(1.28)	5.51^{ab}(1.69)	5.31^{ab}(1.34)	5.52^{ab}(1.56)	5.30^{ab}(1.46)	5.19^{ab}(1.36)	***
C20 Standardized square[h] of leaf area	1.27^{b}(0.31)	1.13^{ab}(0.30)	1.53^{a}(0.29)	1.41^{ab}(0.29)	1.35^{ab}(0.42)	1.58^{a}(0.43)	1.62^{a}(0.34)	1.56^{ab}(0.31)	***
C21 Mean seed length (cm)	0.23^{e}(0.01)	0.23^{de}(0.01)	0.29^{a}(0.01)	0.27^{bc}(0.01)	0.26^{cd}(0.02)	0.26^{bc}(0.02)	0.24^{d}(0.02)	0.28^{b}(0.02)	***
C22 Total number of seeds per capitulum[f]	21.0^{c} (7.9)	29.9^{abc}(19.5)	28.9^{bc} (11.4)	28.6^{abc}(12.7)	36.3^{ab}(14.6)	40.7^{ab}(17.9)	28.5^{ab}(13.8)	40.9^{a}(14.5)	***
C23 Number of pollen pores	2.98^{c}(0.09)	3.0^{bcd}(0)	4.00^{a}(0)	3.35^{b}(0.15)	3.33^{b}(0.28)	3.43^{b}(0.31)	2.70^{d}(0.44)	3.55^{b}(0.46)	***
C26 Time to apical capitulum anthesis (days)[f]	35.0^{c} (5.8)	39.0^{bc}(10.9)	36.3^{c} (7.2)	34.0^{c} (7.4)	44.4^{b}(13.6)	54.4^{a}(18.9)	47.8^{ab}(12.6)	43.2^{ab}(11.7)	***
C27 Number of pedicel bracts[f]	1.39^{ab}(0.49)	1.60^{ab}(0.55)	1.10^{b}(0.30)	1.50^{a}(0.51)	1.37^{ab}(0.57)	1.54^{a}(0.51)	1.19^{ab}(0.51)	1.15^{ab}(0.37)	**
C28 Total number of pollen grains[f]	271.8^{e} (73.6)	451^{abcd}(124)	632.0^{a}(124.4)	605.9^{ab}(138.2)	472.5^{c}(173.3)	488.5^{bc}(161.4)	353.7^{d}(96.1)	570.4^{abc}(163.5)	***
C29 Number of stigmatic papillae[f]	6.1^{d} (2.5)	5.4^{d} (1.9)	21.1^{a} (2.5)	13.9^{bc}(3.9)	14.7^{c} (8.5)	15.6^{bc}(9.3)	11.4^{c} (6.5)	20.6^{ab}(8.7)	***
C30 Midleaf length (cm)	9.5^{c} (1.3)	8.6^{c} (2.0)	15.3^{a} (1.5)	12.6^{b} (1.8)	11.8^{b} (2.2)	12.38^{b}(2.4)	11.6^{b} (1.8)	12.36^{b}(2.1)	***
C31 Apical lobe length (cm)	2.46^{b}(0.68)	2.29^{b}(0.74)	3.31^{a}(0.74)	3.11^{ab}(0.60)	2.72^{b}(0.76)	2.71^{b}(0.83)	3.35^{a}(0.65)	2.74^{ab}(0.85)	***
C32 Apical lobe basal width[f] (cm)	0.68^{c}(0.18)	0.70^{abc}(0.17)	0.78^{bc}(0.17)	0.84^{abc}(0.24)	0.72^{bc}(0.18)	0.78^{bc}(0.21)	0.95^{a}(0.32)	0.89^{ab}(0.19)	***
C33 Mid-lobe vein length[f] (cm)	2.23^{b}(0.64)	1.71^{b}(0.52)	2.84^{a}(0.72)	2.80^{ab}(0.78)	2.52^{ab}(0.78)	2.74^{ab}(0.93)	2.66^{ab}(0.79)	2.37^{ab}(0.64)	**
C34 Mid-lobe lamina width (cm)	0.36^{b}(0.10)	0.28^{b}(0.04)	0.48^{a}(0.10)	0.40^{ab}(0.11)	0.41^{ab}(0.15)	0.49^{a}(0.15)	0.47^{ab}(0.18)	0.55^{a}(0.19)	***
C35 Basal auricle width (cm)	1.33^{b}(0.49)	0.93^{b}(0.13)	2.18^{a}(0.53)	1.39^{b}(0.60)	1.40^{b}(0.72)	1.53^{b}(0.78)	1.47^{b}(0.52)	1.47^{b}(0.65)	***
C36 Mean basal auricle height (cm)	1.79^{ab}(0.49)	1.33^{ab}(0.31)	1.85^{ab}(0.54)	1.51^{b}(0.37)	1.79^{ab}(0.67)	2.00^{ab}(0.65)	1.96^{ab}(0.54)	2.10^{a}(0.55)	*
C37 Basal lamina width[f] (cm)	0.47^{ab}(0.16)	0.39^{ab}(0.02)	0.50^{a}(0.19)	0.35^{b}(0.09)	0.37^{b}(0.23)	0.42^{ab}(0.21)	0.38^{ab}(0.13)	0.45^{ab}(0.16)	•

Table 3.10. Continued

Taxa	*S. vulgaris* v. *vulgaris*	*S. vulgaris* v. *hibernicus*	York radiate groundsel	F_1	F_2 York.	F_2 vulg.	B_1 vulg.	B_1 York.	
N=	33	5	40	18	48	24	21	20	
Character									*P*
C38 Basal auricle extension beyond stem attachment[f] (mm)	1.56^{ab}(0.59)	1.42^{ab}(0.32)	2.06^{c}(0.33)	1.72^{bc}(0.43)	1.65^{b}(0.46)	1.82^{bc}(0.54)	1.23^{a}(0.44)	1.65^{ab}(0.39)	***
C39 Right basal lobe width (cm)	1.26 (0.54)	1.11 (0.52)	1.66 (0.47)	1.38 (0.63)	1.38 (0.60)	1.48 (0.60)	1.51 (0.54)	1.53 (0.62)	ns
C40 Height of left basal lobe (cm)	3.07 (0.57)	2.98 (0.63)	3.29 (0.65)	2.82 (0.76)	3.22 (0.58)	3.58 (0.55)	3.41 (0.65)	3.62 (0.61)	ns
C41 Width of two apical lobes	2.58 (1.06)	2.22 (1.31)	2.73 (1.00)	2.89 (0.83)	2.61 (1.21)	2.72 (1.00)	3.01 (1.03)	2.57 (1.18)	ns
C42 Apical angle A	117.1^{a}(32.7)	123.6^{ab}(15.8)	95.8^{b}(14.3)	118.3^{a}(13.0)	118.5^{a}(28.1)	129.4^{a}(20.1)	128.1^{a}(20.4)	112.9^{ab}(19.9)	***
C43 Apical angle B	93.7^{a}(16.6)	91.9^{a}(13.8)	92.8^{a}(15.8)	104.0^{a}(16.9)	101.7^{a}(20.6)	104.2^{a}(14.3)	99.6^{a}(11.5)	101.6^{a}(16.5)	*
C44 Secondary vein angle of apical adjacent lobe	44.0^{a} (8.4)	36.2^{ab}(3.2)	33.7^{b} (8.1)	34.9^{b} (7.5)	37.9^{b}(10.0)	37.9^{ab}(6.6)	35.3^{b} (5.4)	34.8^{b} (9.6)	***
C45 Mid-lobe secondary vein angle	67.3^{ab}(9.3)	66.9^{ab}(10.9)	73.9^{a} (8.8)	75.4^{a} (7.9)	69.4^{ab}(11.1)	65.5^{b} (7.8)	68.2^{ab}(9.1)	68.5^{ab}(9.6)	**
C46 Mid-lobe apical angle	118.4^{a} (50.3)	97.1^{abc}(47.5)	58.7^{c} (15.8)	83.6^{abc}(19.9)	85.9^{b}(39.2)	97.2^{ab}(51.0)	113.4^{ab}(47.2)	79.1^{bc}(40.5)	***
C47 Basal angle A	101.9^{ab}(23.5)	100.6^{ab}(11.3)	102.4^{ab}(27.6)	105.6^{ab}(27.7)	108.6^{a}(21.0)	107.8^{ab}(23.8)	86.9^{b}(22.6)	96.8^{ab}(25.6)	*
C48 Basal angle B	46.0^{a}(12.6)	39.9^{a} (5.1)	54.9^{a}(13.1)	56.6^{a}(22.3)	46.9^{a}(15.0)	46.9^{a}(13.6)	48.8^{a}(14.8)	45.6^{a}(11.3)	*
Self seed set%[i]	82.4^{b} (0.03)	82.4^{abc}(0.04)	78.5^{a} (0.005)	63.8^{bc}(0.001)	61.7^{c} (0.08)	53.1 (0.04)	66.1 (0.08)	67.2 (0.03)	***
Pollen stainability%[i]	97.6^{a} (0.12)	97.2^{ab}(0.11)	99.6^{ab}(0.08)	97.0^{abc}(0.16)	94.1^{bc}(0.27)	95.3^{c} (0.20)	92.1^{abc}(0.37)	98.4^{abc}(0.18)	***

[f] Log^{e} transformed.
[g] Calculated as midleaf perimeter divided by the square root of area; high ratio indicating highly divided leaf.
[h] Perimeter and square of area measures were divided by midleaf length to standardize.
[i] Arcsine transformed

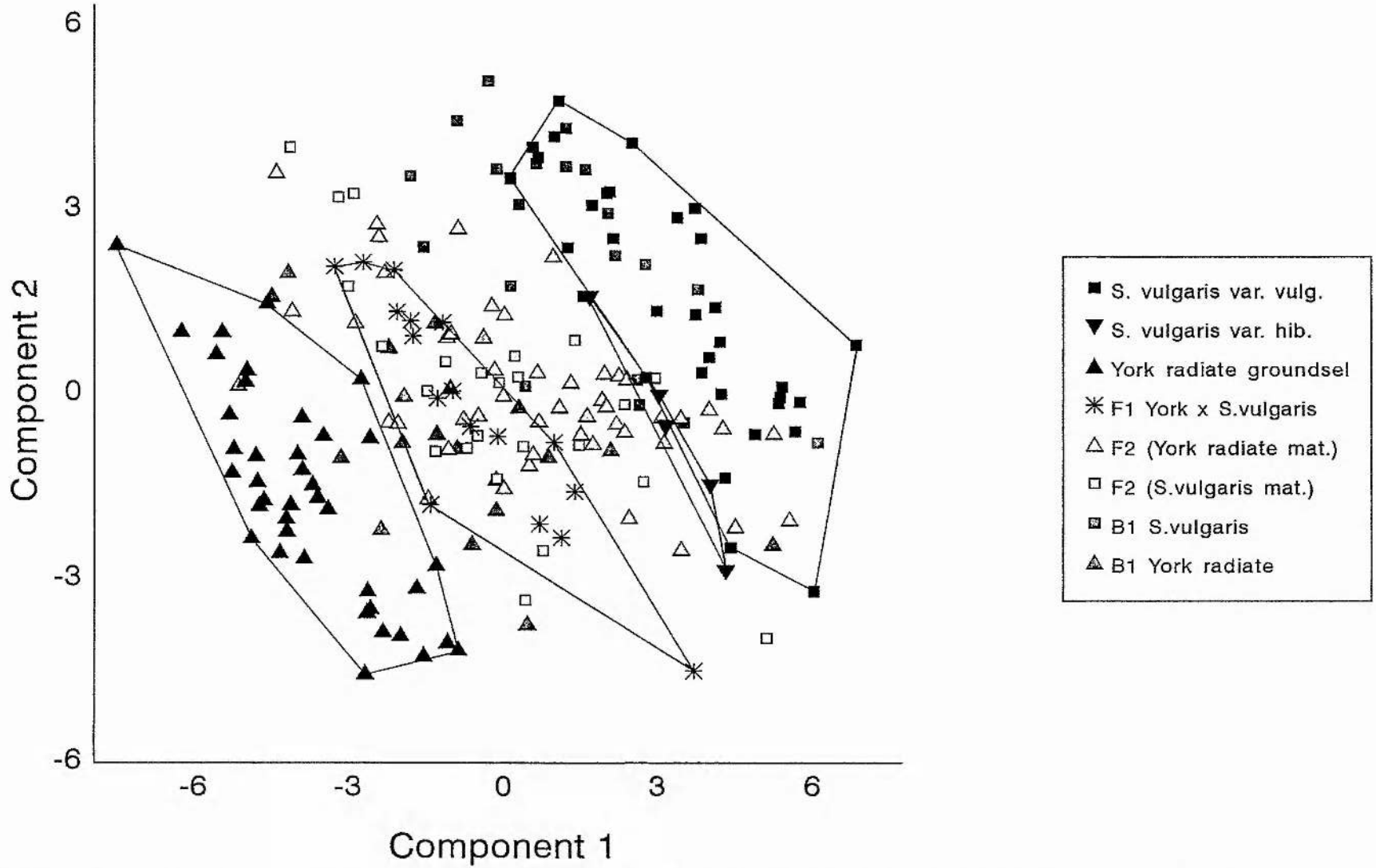


Figure 3.9. Plot of the scores (component 1 vs component 2), following principal component analysis of 39 morphological characters, for parental taxa and selfed and backcrossed progeny of crosses between York radiate groundsel and *S. vulgaris* var. *vulgaris*. The first three eigen values were 9.19, 4.21 and 3.12 and described 22.9, 10.5 and 7.8% of the variance in the data respectively. Characters that contributed most heavily to the first principal component axis (with values of the eigenvectors shown in parentheses) were: C9, Mean calyculus bract length (-0.206); C10, Number of ray florets (-0.212); C11, Mean outer floret length (-0.212); C20, Standardized square of leaf area (-0.217); C19, Standardized leaf perimeter (-0.231); C15, Number of midleaf lobes (-0.246); C18 Leaf dissection (-0.271); C32, Apical lobe basal width (-0.206); C34, Mid lobe lamina width (-0.224). Characters contributing most to principal component axis 2 were; C4, Capitulum length (0.229); C10, Number of ray florets (-0.204); C11, Mean outer floret length (-0.245); C23, Number of pollen pores (-0.214); C21, Mean seed length (0.246); C20, Standardized square of leaf area (0.202); C32, Apical lobe basal width (0.233); C37, Basal lamina width (0.280); C38, Basal auricle extension beyond stem attachment (0.212); C39, Right basal lobe width, (0.226); C41, Width of two apical lobes (0.295).

Table 3.11. Listing of discriminant function morphometric classification of 113 F_1, F_2 and B_1 hybrid progeny produced from crosses between York radiate groundsel and *S. vulgaris*, based on all canonical variate axes, into one of four parental classes significantly differentiated by CVA. The original maternal parent of F_1 crosses are included in parentheses for F_2 progeny.

Discriminant classes	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *hibernicus*	York radiate groundsel	F_1 hybrid	Total
Hybrid progeny					
F_2 (York radiate groundsel)	3	2	4	39	48
F_2 (*S. vulgaris* var. *vulgaris*)	1	1	5	17	24
B_1 to *S. vulgaris* var. *vulgaris*	15	3		3	21
B_1 to York radiate groundsel		1	12	7	20
Total	27	16	23	47	113

vulgaris plants (C18, C19, C20, C30, C31, C33 and C35). For one of the 16 characters for which York radiate groundsel and *S. vulgaris* var. *vulgaris* could not be distinguished, the B_1 and F_2 generations exhibited a mean phenotype that was novel compared to parental taxa (C26). In addition F_2 progeny exhibited a significant reduction in mean pollen fertility (94.5%) and open-seed set (59%) compared to the near full fertility of parent taxa, this interesting observation is examined in more detail in Chapter 4.

When the above two characters were excluded from further analysis it was established that four groups (York radiate groundsel, *S. vulgaris* var. *vulgaris*, var. *hibernicus* and F1 hybrids between *S. vulgaris* var. *vulgaris* and York radiate groundsel) could be differentiated by PCA. The first two axes of the PCA clearly separated plants of *S. vulgaris* var. *vulgaris* and York radiate groundsel into two distinct groups (Figure 3.9), and placed F_1 progeny in an intermediate position between these two taxa, while *S. vulgaris* var. *hibernicus* plants from Edinburgh clustered closely to var. *vulgaris* individuals. Examination of Mahalonobis' distances produced by canonical variate analysis of the 39 morphological character data set, significantly differentiated ($p<0.001$) these four groups. Following comparison of all CVA axes, DFA assigned all 111 F_2 and B_1 hybrid progeny into one of these four groups at the 99.9% significance level (Table 3.11). DFA assigned 12 F_2 plants to the *S. vulgaris* var. *vulgaris* group, 12 to the var. *hibernicus* group, 11 to the York radiate groundsel group and 37 to the F_1 hybrid group. The B_1 progeny were mainly assigned to the taxon to which they had been backcrossed, so 15 of 21 backcrosses to *S. vulgaris* var. *vulgaris* were assigned to the var. *vulgaris* group and 12 of 20 hybrid progeny that were products of backcrossing to York radiate groundsel were assigned to the York radiate groundsel group.

Morphological analysis of hybrid swarm material from Passage West, Cork

Means and standard deviations of 26 morphological characters measured on material of Cork radiate groundsel, *S. vulgaris* var. *hibernicus*, var. *vulgaris* and *S. squalidus*, plus plants of York radiate groundsel and Edinburgh *S. vulgaris* var. *hibernicus* are presented in Table 3.12. Single character one-way ANOVA revealed that Cork radiate groundsel plants had a high number of pollen pores (C23), had longer and broader outer ray florets (C11, C12) and were slower to flower (C26) than *S. vulgaris* var. *hibernicus* from Edinburgh. Also, var. *hibernicus* plants from Cork were shown to be slower to flower (C26) and had a shorter, smaller midleaf (C14, C20) with a more acute apical angle (C16) than Edinburgh plants. Neither Cork radiate groundsel nor *S. vulgaris* var. *hibernicus* from Cork possessed long achenes (C21) nor many

Table 3.12. Means, significant differences (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns-not significant) and results of Tukey-Kramer multiple comparison for 26 morphological traits measured on Cork individuals of *S. squalidus*, *S. vulgaris* var. *vulgaris*, var. *hibernicus* and Cork radiate groundsel, plus individuals of *S. vulgaris* var. *hibernicus* from Edinburgh, and York radiate groundsel. Means sharing the same superscript are not significantly different ($P \leq 0.05$).

Taxa	*S. vulgaris* v. *vulgaris*	*S. vulgaris* v. *hibern.*	*S. vulgaris* v. *hibern.*	Cork radiate	York radiate.	*S. squalidus*	
Location	Cork	Edinburgh	Cork	groundsel	groundsel	Cork	
N=	10	12	10	8	10	9	
Character							*P*
C1 Plant height[f](cm)	26.47^{b}	23.33^{b}	24.90^{b}	26.86^{ab}	20.57^{b}	35.66^{a}	***
C2 Inflorescence length[f](mm)	1.853^{bc}	1.728^{bc}	1.579^{c}	1.839^{bc}	2.215^{ab}	2.653^{a}	***
C3 Pedicel length[f](mm)	0.874^{ab}	0.772^{ab}	0.543^{b}	0.865^{ab}	1.183^{ab}	1.641^{a}	*
C4 Capitulum length[f](mm)	0.979	0.956	1.037	0.974	1.032	1.013	ns
C5 Capitulum width[f](mm)	0.359^{b}	0.382^{b}	0.387^{b}	0.402^{b}	0.398^{b}	0.823^{a}	***
C6 Number of phyllaries	20.10^{ab}	19.25^{ab}	10.40^{a}	20.75^{a}	18.20^{b}	21.00^{a}	**
C7 Proportion of phyllaries with black tips[g]	0.942^{ab}	0.912^{ab}	0.995^{a}	0.856^{ab}	0.274^{c}	0.756^{b}	***
C8 Number of calyculus bracts	11.60^{ab}	11.67^{ab}	13.80^{a}	14.13^{a}	6.80^{c}	8.11^{bc}	***
C9 Mean calyculus bract length[f](mm)	0.249^{c}	0.333^{ab}	0.339^{ab}	0.356^{ab}	0.396^{a}	0.294^{bc}	***
C10 Number of ray florets	0.00^{c}	11.67^{a}	11.00^{a}	11.25^{a}	8.70^{b}	11.67^{a}	***
C11 Mean outer floret length (mm)	0.000^{d}	0.557^{c}	0.649^{bc}	0.719^{b}	0.501^{c}	1.404^{a}	***
C12 Mean outer floret width (mm)	0.000^{d}	0.145^{c}	0.173^{bc}	0.189^{b}	0.143^{c}	0.375^{a}	***
C13 Longest leaf length[f](cm)	13.39^{bc}	16.17^{ab}	12.88^{bc}	15.35^{ab}	18.18^{a}	11.22^{c}	***
C14 Midleaf length[f](cm)	9.33^{bc}	10.32^{b}	6.96^{d}	8.06^{bcd}	13.70^{a}	7.64^{cd}	***
C15 Number of midleaf lobes	12.80^{b}	11.92^{bc}	13.00^{b}	12.87^{b}	16.80^{a}	11.00^{c}	***
C16 Midleaf apical angle (degrees)	146.0^{a}	137.9^{a}	109.1^{bc}	119.8^{ab}	100.6^{bc}	85.9^{c}	***
C17 Midleaf secondary vein angle (degrees)	62.15^{a}	66.21^{a}	61.10^{a}	57.75^{a}	57.85^{a}	38.44^{c}	***
C18 Leaf dissection[h]	5.537^{c}	7.670^{ab}	7.901^{ab}	8.346^{ab}	9.371^{a}	6.662^{bc}	***
C19 Standardized leaf perimeter[i]	2.275^{b}	3.021^{ab}	2.426^{b}	3.050^{ab}	3.537^{a}	2.632^{b}	***
C20 Standardized square of leaf area[i]	0.409^{a}	0.392^{a}	0.312^{b}	0.386^{ab}	0.377^{ab}	0.390^{a}	**
C21 Seed length[f](mm)	0.214^{c}	0.245^{b}	0.233^{bc}	0.238^{bc}	0.295^{a}	0.229^{bc}	***
C22 Total number of seeds per capitulum	55.50^{ab}	52.42^{b}	45.30^{b}	54.13^{ab}	63.80^{a}	66.11^{a}	***
C23 Number of pollen pores[f]	3.05^{c}	3.00^{c}	3.00^{c}	3.44^{b}	4.00^{a}	3.00^{c}	***
C24 Pollen pore size[f] (graticule units 40=0.1mm)	2.142	2.167	2.206	2.288	1.954	2.461	ns
C25 Proportion of self seed set per capitulum [g]	0.786^{ab}	0.774^{ab}	0.776^{ab}	0.679^{b}	0.862^{a}	0.005^{c}	***
C26 Time to apical capitulum anthesis (days)f	75.90^{cd}	70.83^{d}	90.10^{ab}	82.50^{bc}	71.20^{d}	96.67^{a}	***

[f] Log^{e} transformed. [g] Arcsine transformed.

[h] Calculated as midleaf perimeter divided by the square root of area; high ratio indicating highly divided leaf.

[i] Perimeter and square of area measures were divided by midleaf length to standardize.

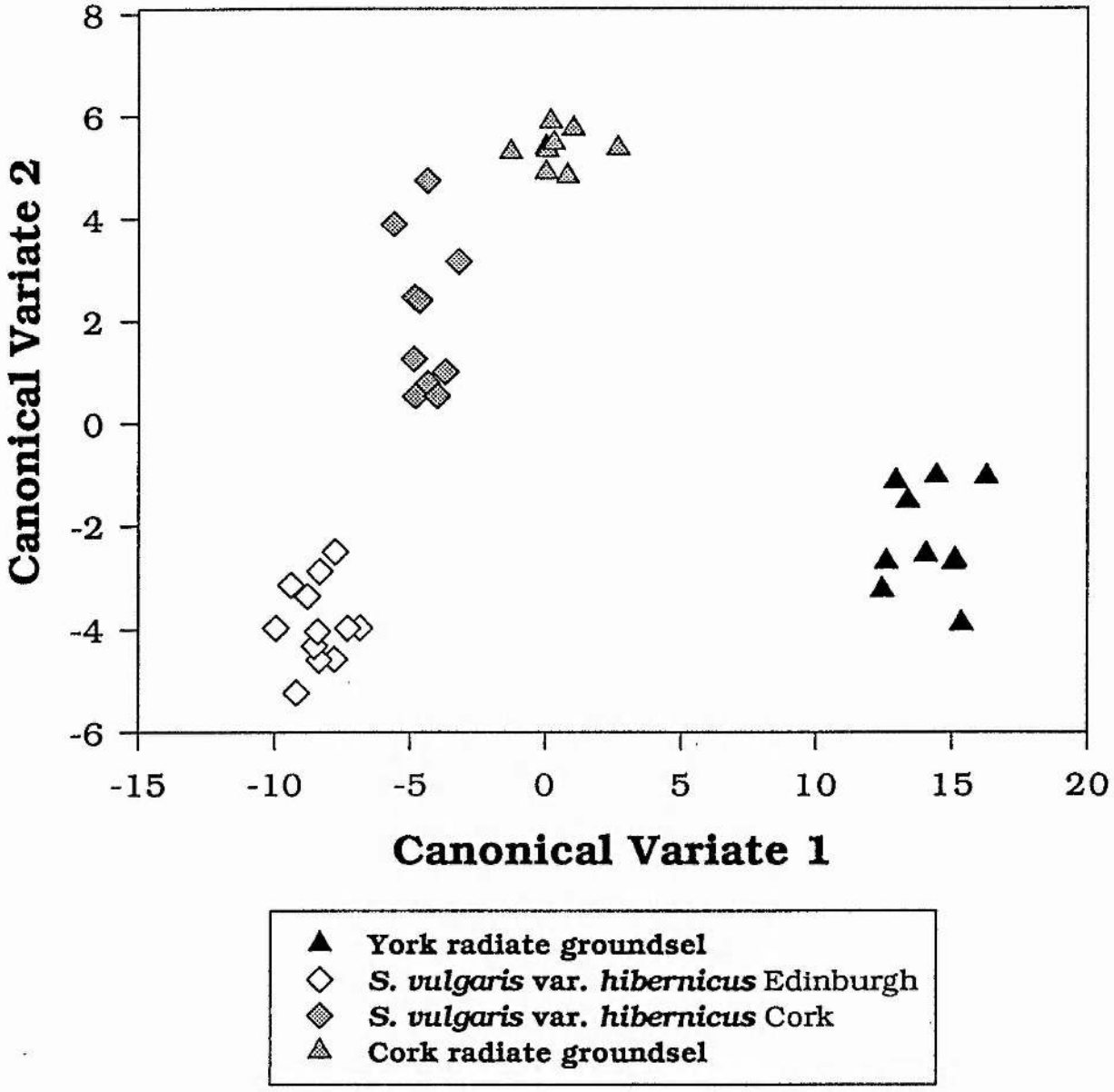


Figure 3.10. A plot of the scores (CV1 vs CV2) for each individual within a given taxon/population following canonical variate analysis of 26 morphometric characters; the first two canonical variables were statistically significant ($P < 0.001$), and accounted for 81.2% and 14.2% of the total variance in measurements respectively. The following characters had a high weighting on canonical variable one (total-sample standardized canonical coefficients shown in parentheses): C2, infloresence length (-3.004); C3, number of pedicel bracts (4.283); C11, mean outer floret length (3.382); and C12, width (-4.117); C18, leaf dissection (5.500); C26, time to apical anthesis (4.731); C19, standardized midleaf perimeter (-3.651); and C20, standardized square of midleaf area (4.718). Characters contributing most to the second canonical variable were C18 (3.938) and C20 (3.184).

lobed leaves (C15), as did York radiate groundsel individuals, but all three taxa had significantly longer calyculus bracts (C9) and more dissected leaves (C18) than *S. vulgaris* var. *vulgaris*.

Canonical variate analysis of the four radiate groundsel populations separated Edinburgh *S. vulgaris* var. *hibernicus* plants from individuals of York radiate groundsel along canonical variate one (Figure 3.10). Cork radiate groundsel material was also separated from the grouping of *S. vulgaris* var. *hibernicus* from Cork by the first canonical variate and was placed in an intermediate position between York radiate groundsel and Edinburgh *S. vulgaris* var. *hibernicus*. *S. vulgaris* var. *hibernicus* plants from Cork were intermediate between the grouping of Cork radiate groundsel and Edinburgh *S. vulgaris* var. *hibernicus*. The second canonical variate further separated the two groupings of Cork material from York radiate groundsel and Edinburgh *S. vulgaris* var. *hibernicus* individuals. It was evident from the analysis that all individuals of Cork radiate groundsel were morphologically disparate from York radiate groundsel based on canonical variate analysis, and single character analysis confirmed that a number of morphological characters distinguished the two taxa.

Geographic and genetic differentiation of groundsel populations from York

The current distribution of York radiate groundsel populations in York (Figure 3.4) is much reduced to that noted previously (Figure 3.2), but thriving populations still exist at Dalton Terrace (site 1) and around Lendal Bridge (sites 4 and 5). The frequency of allozyme phenotypes exhibited by radiate and non-radiate forms of groundsel found at these sites in 1993 (or 1991) and 1994 are listed in Table 3.13. It was notable that most York radiate groundsel plants surveyed exhibited an identical isozyme phenotype over all enzyme systems examined. This observation was supported by UPGMA analysis of genetic distances between samples based on allozyme phenotype frequencies (Figure 3.11). This showed that there was no genetic differentiation between any samples of York radiate groundsel. Samples of *S. vulgaris* var. *vulgaris*, on the other hand, showed considerable variation. At Dalton Terrace (site 1), most var. *vulgaris* individuals exhibited the *Gdh-1aa* phenotype, which was present in this taxon at very low frequency at almost all other sites. Similarly, most plants of *S. vulgaris* var. *vulgaris* samples from the Spotted Cow public house (site 9) exhibited the β*Est-1a* phenotype, that was absent from nearly all other *S. vulgaris* individuals surveyed. The β*Est-1a* phenotype is normally considered to be diagnostic of York radiate groundsel (Irwin and Abbott, 1992), and it was of interest that *S. vulgaris* var. *vulgaris* plants in the Spotted Cow sample often exhibited a leaf morphology very

Table 3.13. Allozyme phenotype frequencies in samples of *S. vulgaris* var. *vulgaris*, York radiate groundsel and *S. squalidus* from York (see figure 3.4). Taxon abbreviations; SVNR=*S. vulgaris* var. *vulgaris*, SVRR=*S. vulgaris* var. *hibernicus*, SVYRR=York radiate groundsel, S.squal=*S. squalidus*, SVYNR=*S. vulgaris* var. *vulgaris* with York radiate groundsel-like leaves.

				aEst-1				BEst-3				Idh-1		BEst-1	
Taxa	Location	Year	N=	aa	ab	bb	nn	bb	bc	cc	a*b	ab	bb	aa	nn
SVNR	1 Dalton Terrace	1993	71	1.00	-	-	-	-	-	1.00	-	1.00	-	0.01	0.99
SVNR	1 Dalton Terrace	1994	37	1.00	-	-	-	-	-	1.00	-	1.00	-	-	1.00
SVYRR	1 Dalton Terrace	1993	117	1.00	-	-	-	-	-	1.00	-	1.00	-	0.97	0.03
SVYRR	1 end Dalton Terrace	1993	1	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	1 Dalton Terrace	1994	10	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
S.squal	1 end Dalton Terrace	1993	1	-	-	-	1.00	-	1.00	-	-	-	1.00	1.00	-
SVNR	2 NW Lendal Bridge	1993	27	1.00	-	-	-	-	-	1.00	-	1.00	-	-	1.00
SVRR	2 NW Lendal Bridge	1993	8	1.00	-	-	-	-	-	1.00	-	1.00	-	0.12	0.88
SVNR	2 NW Lendal Bridge	1994	1	1.00	-	-	-	-	-	1.00	-	1.00	-	-	1.00
S.squal	2 NW Lendal Bridge	1993	9	-	-	-	1.00	-	1.00	-	-	-	1.00	1.00	-
SVNR	3 SW Lendal Bridge	1993	6	1.00	-	-	-	0.33	-	0.67	-	1.00	-	-	1.00
SVNR	3 SW Lendal Bridge	1994	22	0.95	-	0.05	-	0.36	-	0.64	-	1.00	-	-	1.00
SVYRR	3 SW Lendal Bridge	1993	1	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	3 SW Lendal Bridge	1994	1	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
S.squal	3 SW Lendal Bridge	1993	5	-	-	-	1.00	-	1.00	-	-	-	1.00	1.00	-
SVNR	4 SE Lendal Bridge	1994	6	0.66	0.17	0.17	-	-	0.17	0.83	-	1.00	-	-	1.00
SVNR	5 Lendal Bridge park	1994	10	1.00	-	-	-	0.10	-	0.90	-	1.00	-	-	1.00
SVNR	6 Victoria House CP	1993	14	1.00	-	-	-	-	-	1.00	-	1.00	-	-	1.00
SVNR	Ouse Bridge	1993	8	1.00	-	-	-	-	-	1.00	-	1.00	-	-	1.00
SVYRR	4 SE Lendal Bridge	1991	56	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	4 SE Lendal Bridge	1994	19	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	5 Lendal Bridge park	1994	3	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	6 Victoria House CP	1993	1	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	7 All Saints church	1993	1	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
SVYRR	8 Viking Hotel	1994	1	1.00	-	-	-	-	-	1.00	-	1.00	-	1.00	-
S.squal	7 All Saints church	1993	1	-	-	-	1.00	-	1.00	-	-	-	1.00	1.00	-
S.squal	Ouse Bridge	1993	1	-	-	-	1.00	-	1.00	-	-	-	1.00	1.00	-
SVNR	9 Spotted cow pub	1993	8	1.00	-	-	-	-	-	1.00	-	1.00	-	0.13	0.87
SVYNR	9 Spotted cow pub	1993	18	1.00	-	-	-	-	-	1.00	-	1.00	-	0.72	0.28
S.squal	9 Spotted cow pub	1993	1	-	-	-	1.00	-	1.00	-	-	-	1.00	1.00	-

Table 3.13. Continued.

			Aat-2						Gdh-1			Aco-1			Acp-2		
Taxa	Location	Year	ab	bb	bc	cc	ac	abc	aa	ab	bb	aa	bb	cc	aa	ab	bb
SVNR	1 Dalton Terrace	1993	1.00	-	-	-	-	-	0.80	0.06	0.14	0.99	0.01	-	1.00	-	-
SVNR	1 Dalton Terrace	1994	1.00	-	-	-	-	-	0.84	0.13	0.03	1.00	-	-	1.00	-	-
SVYRR	1 Dalton Terrace	1993	1.00	-	-	-	-	-	0.06	0.02	0.92	0.98	0.02	-	1.00	-	-
SVYRR	1 end Dalton Terrace	1993	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
SVYRR	1 Dalton Terrace	1994	1.00	-	-	-	-	-	-	0.10	0.90	1.00	-	-	1.00	-	-
S.squal	1 end Dalton Terrace	1993	-	-	-	1.00	-	-	1.00	-	-	-	-	1.00	-	-	1.00
SVNR	2 NW Lendal Bridge	1993	0.89	-	0.11	-	-	-	-	-	1.00	-	1.00	-	1.00	-	-
SVRR	2 NW Lendal Bridge	1993	0.12	-	-	-	0.88	-	0.12	-	0.88	0.12	0.88	-	1.00	-	-
SVNR	2 NW Lendal Bridge	1994	1.00	-	-	-	-	-	-	-	1.00	-	1.00	-	1.00	-	-
S.squal	2 NW Lendal Bridge	1993	-	0.67	0.33	-	-	-	1.00	-	-	-	-	1.00	-	-	1.00
SVNR	3 SW Lendal Bridge	1993	1.00	-	-	-	-	-	-	-	1.00	0.33	0.67	-	1.00	-	-
SVNR	3 SW Lendal Bridge	1994	1.00	-	-	-	-	-	-	-	1.00	0.32	0.68	-	1.00	-	-
SVYRR	3 SW Lendal Bridge	1993	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
SVYRR	3 SW Lendal Bridge	1994	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
S.squal	3 SW Lendal Bridge	1993	-	-	0.60	0.40	-	-	1.00	-	-	-	-	1.00	-	-	1.00
SVNR	4 SE Lendal Bridge	1994	1.00	-	-	-	-	-	-	-	1.00	0.17	0.83	-	1.00	-	-
SVNR	5 Lendal Bridge park	1994	1.00	-	-	-	-	-	0.50	0.30	0.20	0.60	0.30	0.10	1.00	-	-
SVNR	6 Victoria House CP	1993	1.00	-	-	-	-	-	-	-	1.00	-	1.00	-	1.00	-	-
SVNR	Ouse Bridge	1993	1.00	-	-	-	-	-	-	-	1.00	0.13	0.87	-	1.00	-	-
SVYRR	4 SE Lendal Bridge	1991	0.91	0.09	-	-	-	-	-	-	1.00	0.96	0.04	-	1.00	-	-
SVYRR	4 SE Lendal Bridge	1994	1.00	-	-	-	-	-	-	-	1.00	0.89	0.11	-	1.00	-	-
SVYRR	5 Lendal Bridge park	1994	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
SVYRR	6 Victoria House CP	1993	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
SVYRR	7 All Saints church	1993	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
SVYRR	8 Viking Hotel	1994	1.00	-	-	-	-	-	-	-	1.00	1.00	-	-	1.00	-	-
S.squal	7 All Saints church	1993	-	1.00	-	-	-	-	1.00	-	-	-	-	1.00	-	-	1.00
S.squal	Ouse Bridge	1993	-	1.00	-	-	-	-	1.00	-	-	-	-	1.00	-	-	1.00
SVNR	9 Spotted cow pub	1993	1.00	-	-	-	-	-	0.13	-	0.87	0.25	0.75	-	1.00	-	-
SVYNR	9 Spotted cow pub	1993	1.00	-	-	-	-	-	-	-	1.00	0.06	0.94	-	1.00	-	-
S.squal	9 Spotted cow pub	1993	-	-	1.00	-	-	-	1.00	-	-	-	-	1.00	-	-	1.00

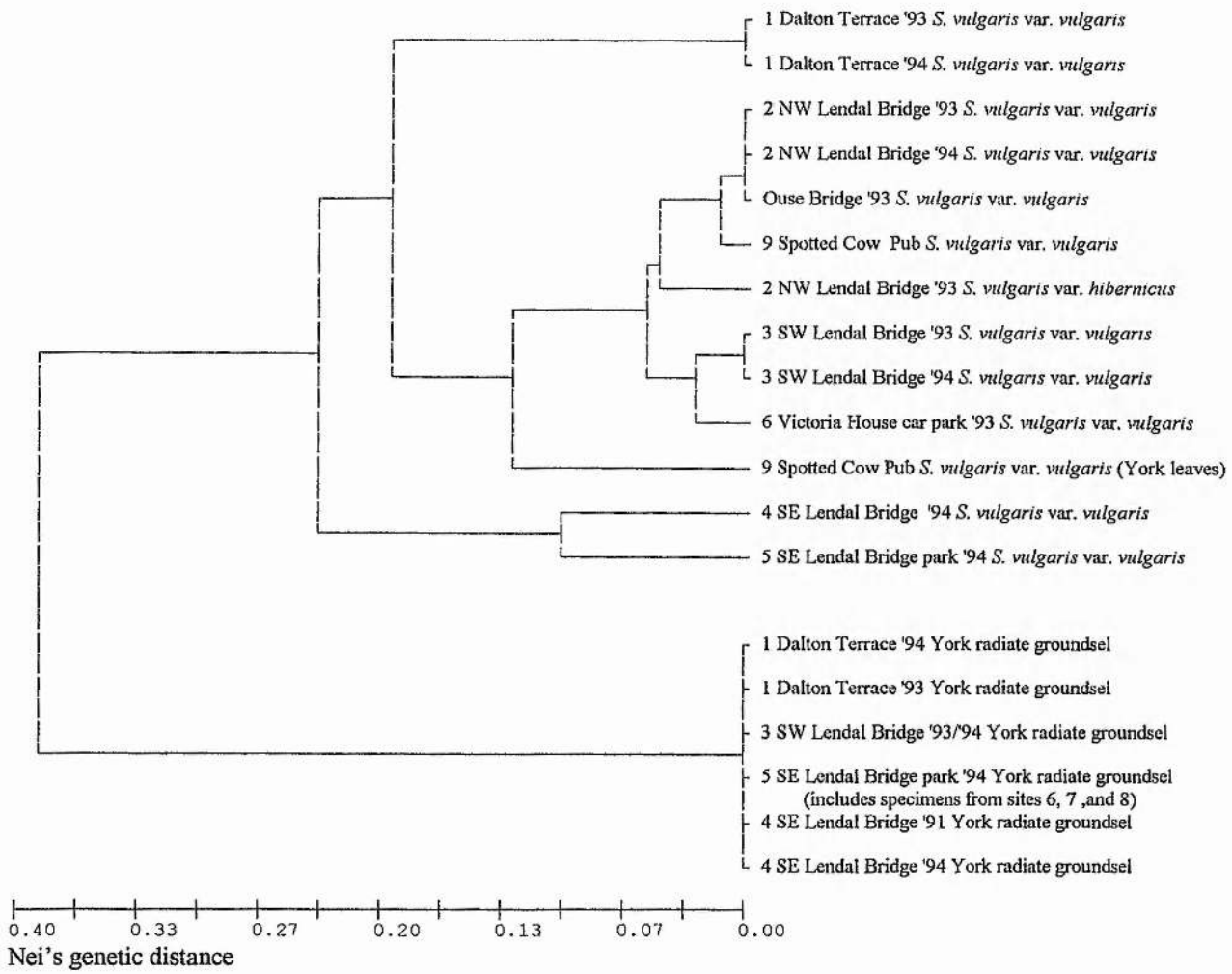


Figure 3.11 Dendrogram produced by UPGMA plot of Nei's genetic distance (1972) calculated from isozyme phentoype frequency data presented in Table 3.3. See figure 3.4 for location of sites.

similar to that of York radiate groundsel, but were rayless. These plants were also well differentiated from York radiate groundsel populations in the UPGMA dendrogram. Other plants from the Spotted Cow site were more typical of *S. vulgaris* in morphology and lacked the β*Est-1a* allele. These plants clustered with the main group of *S. vulgaris* populations in the UPGMA dendrogram. Material of *S. vulgaris* var. *hibernicus* from North West Lendal Bridge (site 2) clustered with the majority of var. *vulgaris* samples (sites 2, 3, 6 and 9), indicating a closer relationship to this taxon than to York radiate groundsel.

Discussion

Resynthesis of plants similar to York radiate groundsel

Crisp (1972) identified five possible pathways by which tetraploid radiate groundsel progeny could be generated. Pathways 1 and 2 were as follows:

1. Hybridization between *S. cambrensis* and *S. squalidus*. However, Ingram and Noltie (1987) found that the tetraploid hybrid produced from this cross showed a high level of meiotic irregularity and was highly sterile (see also Benoit, Crisp and Jones, 1975), and also has never been reported from the wild (Ingram and Noltie, 1995).

2. A reduction in chromosome number from the hexaploid *S. cambrensis* to a tetraploid radiate groundsel plant. Crisp (1972) reported that after Vosa had planted out a fertile, near hexaploid (2n=57) plant, which resembled *S. cambrensis*, in the Oxford Botany School Gardens, near tetraploid, radiate groundsel plants were found at the site two years later. Crisp considered that chromosome loss through meiotic mispairing had given rise to the tetraploid radiate plants from the near hexaploid. Alternatively, a reduction in chromosome number from hexaploid to near tetraploid could occur following backcrossing to *S. vulgaris*. Ingram and Noltie (1987) demonstrated that a pentaploid hybrid produced by crossing *S. cambrensis* with *S. vulgaris* was highly fertile, although such backcross products have never been found in the wild (Ingram and Noltie, 1995).

As *S. cambrensis* has never been reported from the York area, pathways 1 and 2 were ruled out as routes of origin of York radiate groundsel. The other three possible routes of origin proposed by Crisp (1972) were:

3. Following a cross between tetraploid *S. squalidus* and *S. vulgaris*. Tetraploid *S. squalidus* has never been recorded from the wild which makes this pathway extremely unlikely; however, a lack of examples may only reflect a lack of investigation. It should be noted that tetraploid *S. squalidus* plants generated by colchicine treatment in this chapter, had a number of 'gigas' morphological characters associated with polyploidy (also noted by Crisp, 1972; Housten, 1983; and Taylor, 1984), that would easily distinguish them in a population of diploid *S. squalidus* individuals (e.g. much larger, robust plants, ligules 50% longer than normal *S. squalidus*, elongated internodes and much larger, dissected leaves). It is hard to believe that such morphologically distinct plants would escape the eye of botanists should they occur in the wild.

4. Following fusion of an unreduced gamete of *S. squalidus* with a reduced gamete of *S. vulgaris*. Taylor (1984) has shown that a partially fertile tetraploid hybrid, which she presumed had been generated via this route, produced progeny that were very

similar to *S. vulgaris* var. *hibernicus*. From diagrams in her thesis, it is clear that some of these plants also exhibited a leaf morphology very similar to that of York radiate groundsel.

5. Hybridization between *S. vulgaris* and *S. squalidus*, followed by backcrossing of the triploid F_1 hybrid to *S. vulgaris*, or segregation in the F_2, with resumption of tetraploidy. Ingram, Weir and Abbott (1980) have already shown that fertile, tetraploid plants similar to *S. vulgaris* var. *hibernicus* may be generated by backcrossing the triploid hybrid to *S. vulgaris*, presumably due to the fusion of a balanced gamete (n=20) from the hybrid with a normal gamete produced by *S. vulgaris*.

All three of these routes of origin were examined in the present study. Routes 3 and 4 test the hypothesis that successful hybridization between taxa of different ploidy levels can be achieved by the production of unreduced gametes by the diploid taxon followed by fusion with normal, reduced gametes of the tetraploid taxon (Harlan and deWet, 1975; Bretagnolle and Thompson, 1995). This was invoked as a possible origin of York radiate groundsel by Irwin and Abbott (1992). Route 5 tests the alternative hypothesis that the triploid hybrid acts as a bridge between ploidy levels through an ability to produce genomically stable polyploid gametes (Ratter, 1972, 1973a 1973b; Ingram, 1978; Taylor, 1984; Bretagnolle and Thompson, 1995) that form fertile progeny following backcrossing to the polyploid parent (see Ingram, Weir and Abbott, 1980) or through fusion with each other in the production of the F_2.

Tetraploid F_1 hybrids generated by routes 3 and 4 were very similar to each other in morphology and were partially fertile (pollen stainability 79.5%, open seed set 20.7%), and plants exhibited a number of 'gigas' and hybrid characters, including long rays (10 mm), long achenes (3 mm) and highly dissected leaves. The segregating F_2 and backcrosses to *S. vulgaris*, produced plants that exhibited a wide range of morphological phenotypes. The plotting of individual PCA scores and assigning of hybrid progeny into parental classes by DFA and CVA provided a powerful test of the morphological affinity of hybrid progeny to established taxa. Some of the hybrid progeny were very similar in morphology to York radiate groundsel (16 plants of 116 examined), while others were similar to *S. vulgaris* var. *hibernicus* (39 plants of 116), of those, nearly three times as many were generated in B_1 generations (41 plants) as in the F_2 (14 plants).

The five triploid F_1 hybrids produced by route 5 were highly sterile and only two germinable seed were collected from open pollinated capitula, no seed was produced from backcrosses to *S. vulgaris*. One F_2 plant, derived from a cross in which var. *hibernicus* was the maternal parent, was highly sterile with shrivelled, non-functional anthers and set no seed even under conditions of open pollination. This individual was of interest however, because it exhibited an unusual phenotype, being 'elongated' in appearance, with long internodes and long thin ray florets and leaves, and from descriptions in Crisp's thesis (1972), was similar in morphology to Harland's male sterile dwarf strap plants. The other F_2 plant, derived from a cross in which var. *vulgaris* was the maternal parent, was a partially fertile (pollen stainability 71.1% and open seed set 54.9%), radiate plant that was very similar in overall phenotype to a robust example of *S. vulgaris* var. *hibernicus*. This plant was presumed to be near tetraploid as it was highly interfertile with *S. vulgaris* and must have been produced following chromosome assortment and segregation in F_1 gametes. Of 25 F_3 and B_1 progeny produced from this partially fertile F_2 hybrid, seven were found to be similar in morphology to York radiate groundsel while seven were similar to var. *hibernicus*. Of these 14 hybrid progeny, only three were produced in the F_3, whereas 11 were produced following backcrossing to *S. vulgaris*.

In summary, hybrid progeny similar in morphology to York radiate groundsel and *S. vulgaris* var. *hibernicus* were produced by all three routes of origin examined. However, it was notable that B_1 progeny were always much more likely to be similar to one of these two taxa than were F_2 or F_3 progeny. Further backcrossing of such hybrid progeny to *S. vulgaris* would probably be necessary to produce plants that were identical to *S. vulgaris* var. *hibernicus* in morphology and genotype, but is probably not required to produce the more intermediate phenotype of York radiate groundsel. Although hybrid progeny similar to York radiate groundsel were produced by all three pathways examined, we may wonder which of these three is likely to have been the route of origin of York radiate groundsel in the wild. As already pointed out, route 3 may be excluded as a possibility due to previous failure to find tetraploid *S. squalidus* growing in natural populations of the species. Thus we are left with routes 4 and 5.

Route 4 depends on the ability of diploid *S. squalidus* to produce unreduced gametes that fuse with normally reduced gametes of *S. vulgaris*. Only two tetraploid F_1 hybrids are known to have been generated following fusion of an unreduced gamete from diploid *S. squalidus* with a reduced gamete from *S. vulgaris*. These were produced in work by Taylor (1984) and by work reported in this chapter. Taylor (1984) recorded that out of an unspecified number of crosses attempted, one triploid

and one tetraploid F_1 hybrid was produced, while in the present study, out of six F_1 hybrids generated, five were triploid and one was tetraploid. In all other studies that have crossed *S. squalidus* with *S. vulgaris*, only triploid F_1 hybrids have been generated (Gibbs, 1971; Ingram, 1977 and Ingram, Weir and Abbott, 1980). Although tetraploid F_1 hybrids are less frequently produced than triploid hybrids from crosses between diploid *S. squalidus* and tetraploid *S. vulgaris*, they exhibit much higher fertility. Thus route 4 would seem a likely pathway for the origin of York radiate groundsel.

It is important to note that in this study and that of Taylor's (1984), the spontaneous production of unreduced gametes in *S. squalidus* was not observed, but only inferred from the generation of a hybrid product that must have involved an unreduced gamete. Crisp (1972) noted that *S. squalidus* occasionally produced large pollen grains (0.18%; from a count of 9 in 4959 grains), which were presumed to contain an unreduced chromosome complement. Although Crisp's estimate seems low, there is some evidence that unreduced pollen may fair better under competitive conditions in the stigma than reduced pollen (Mulinix and Lezzoni, 1988) and so frequency may not reflect fertilization success. Recent reviews have shown that the production of unreduced gametes by diploid taxa has played an important role in the evolution of many auto- and allopolyploid species (Harlan and deWet, 1975; Thompson and Lumaret, 1992; Bretagnolle and Thompson, 1995). The production of unreduced gametes is likely to have been important in the origin of *S. cambrensis*, and may have also been instrumental in the origin of *S. vulgaris* var. *hibernicus* and York radiate groundsel.

Route 5 involving the production of balanced diploid gametes by the F_1 hybrid has been the most favoured pathway of origin of radiate groundsel in the past. The validity of this pathway was reinforced when plants similar to *S. vulgaris* var. *hibernicus* were synthesized by backcrossing *S.* x *baxteri* to var. *vulgaris* (Ingram, Weir and Abbott, 1980). However the production of fertile progeny via this route requires two steps; first, the production of *S.* x *baxteri* and second, the production of later generation, fertile progeny either via backcrossing or segregation.

The best estimate of the frequency of *S.* x *baxteri* production in natural populations comes from Marshall and Abbott (1980), who noted that the hybrid occurs regularly but at low frequency. Several studies have examined the potential of experimentally synthesized *S.* x *baxteri* to produce fertile progeny; however, no field estimates are available. Ingram (1977) and Taylor (1984) were not able to obtain any germinable

seed from triploid F_1 hybrids when they were backcrossed to *S. vulgaris* or left to open pollinate. Other workers were more successful and Crisp (1972) reported that Vosa obtained a hexaploid plant from a triploid F_1 hybrid. Ingram, Weir and Abbott (1980) produced two near tetraploids (two others died before flowering) from a single *S.* x *baxteri* plant that had been backcrossed to *S. vulgaris* var. *vulgaris*, but no seed was set when the plant was selfed. Ingram (1978) managed to produce eight partially fertile (seed set 30-55%), near tetraploid (2n=40-44) progeny when she backcrossed a triploid F_1 hybrid to *S. vulgaris* var. *vulgaris*. On selfing the F_1 hybrid, one near diploid (2n=22) plant was obtained while 11 partially fertile F_2 progeny (ranging from hexaploid to tetraploid) were produced following open pollination. In the study reported in this chapter, one partially fertile, presumed tetraploid hybrid was raised from approximately 25,000 *S.* x *baxteri* open pollinated achenes from five *S.* x *baxteri* plants. Three out of the five triploid hybrids failed to produce any germinable seed at all and another one produced only one F_2 progeny that was totally sterile. Although the production of fertile, near tetraploid progeny from triploid F_1 hybrids varies greatly between studies and between plants within a study, it is possible, but likely to be a rare event in the wild. Consequently, we may conclude that route 5 may be less likely to have been the route of origin of York radiate groundsel relative to route 4.

Potential for York radiate groundsel to backcross to *S. squalidus*

Triploid F_1 hybrids between York radiate groundsel and *S. squalidus* exhibited higher seed set (0.63%) and were easier to generate, than *S. vulgaris* x *S. squalidus* triploid hybrids (seed set 0.0088%). F_2 progeny and backcrosses to *S. vulgaris* were easy to produce, exhibited segregation of morphological characters and some were highly fertile (e.g. one B_1 plant exhibited mean seed set of 56.9% and pollen stainability of 98.5%). An examination of meiotic chromosome pairing in one York radiate groundsel x *S. squalidus* triploid F_1 hybrid showed that, on average, two trivalents and four to eight univalents were observed, with the remaining chromosomes forming bivalents. This was in contrast to *S. vulgaris* x *S. squalidus* F_1 hybrids in which no trivalents normally form (Weir and Ingram, 1980). Ratter (1973b) found that trivalent formation can be an indicator of genome homology, and Ingram (1977) noted that genomic relationship may affect the ease with which taxa can be crossed. Taken together, meiotic chromosome paring behaviour and crossing success are consistent with morphological, isozyme and molecular evidence (Chapter 2; Irwin and Abbott, 1992), and suggest that York radiate groundsel exhibits a genomic constitution that is more similar to *S. squalidus* than does *S. vulgaris*. One consequence of the closer genomic relationship between *S. squalidus* and York radiate groundsel is that crossing in the wild may occur at a higher frequency than between *S. vulgaris* and *S. squalidus*

and may offer the potential for further introgression of *S. squalidus* genes into York radiate groundsel.

Morphometric analysis of Cork hybrid material

Morphometric analysis of Cork radiate groundsel individuals from a hybrid swarm at Passage West, Cork, demonstrated that they were distinct from York radiate groundsel and Edinburgh *S. vulgaris* var. *hibernicus* plants. *S. vulgaris* var. *hibernicus* plants from Passage West were found to be intermediate in mean phenotype between Cork radiate groundsel individuals and *S. vulgaris* var. *hibernicus* plants from Edinburgh. This evidence suggests that York radiate groundsel could not have been derived from material occurring in the Passage West hybrid swarm, and that Cork radiate groundsel plants are probably the product of a separate hybridization event to that which gave rise to York material.

The *S. vulgaris* var. *hibernicus* material from Passage West exhibited a wide range of morphological variation and field observations suggest that hybrid swarm material was backcrossing to *S. vulgaris*. At Bristol, Cardiff, Cork and Kings Lynn, intermediate hybrid plants have been identified as components of hybrid swarms (Table 3.2) or 'introgression sequences' (Crisp, 1972). At these locations some intermediate hybrid herbarium specimens appeared similar in overall morphology to York radiate groundsel plants, while others that were closer in morphology to *S. vulgaris*, appeared more similar to Cork radiate groundsel material.

Geographic and genetic differentiation of York radiate groundsel populations

York radiate groundsel plants were mainly found at two sites in York, Dalton Terrace and around Lendal Bridge. Radiate groundsel plants have been recorded in large populations along the banks of the River Ouse and at other locations in York in the late 1980s, but redevelopment of these areas has reduced the number of suitable sites. Despite this, York radiate groundsel is now well established at its current locations.

The two main populations of York radiate groundsel could not be differentiated by isozyme analysis as was the case for all other York radiate groundsel individuals from sites around York. It seems likely that all populations of York radiate groundsel are derived from one founder population and probably from a single origin (also see Chapter 2). In contrast, several York populations of *S. vulgaris* var. *vulgaris* could be distinguished by analysis of isozyme variation. A small group of non-radiate *S. vulgaris* individuals near the Spotted Cow public house appeared morphologically similar to York radiate groundsel, and possessed the *βEst-1a* genotype typically exhibited by York radiate groundsel. J. Warren (personal communication) recalled

that this area was the site of a fairly large population of York radiate groundsel in the late 1980s, until the site was redeveloped into the Barbican Centre. It would appear that these particular non-radiate groundsel plants are derived from this previous population of York radiate groundsel that have lost their ray floret expression, probably due to backcrossing with *S. vulgaris*.

In 1993 only five plants at Dalton Terrace (and none at any other sites) exhibited an isozyme phenotype that suggested hybridization had occurred between *S. vulgaris* and York radiate groundsel. In 1994 no such plants were found at this site. In addition, there was very little evidence of disruption to the typical morphological phenotype of York radiate groundsel in the field, although experimental backcrosses to *S. vulgaris* demonstrated that character coherence was affected. The possibility that breeding barriers exist between the two taxa is examined in more detail in Chapter 4.

Chapter 4.
Maintenance of York radiate groundsel in the wild: Breeding barriers with parental taxa

Introduction

Once a recently arisen hybrid overcomes any sterility barriers associated with its origin, and its genomic constitution is stable enough such that it breeds true to type, other problems may obstruct its establishment and maintenance in the wild. One of the main factors is that of backcrossing to parental taxa that can erode the genetic constitution of the hybrid, and if no isolating mechanisms are present (summarised in Table 4.1), hybridization may lead to introgression of genes between parental taxa (Rieseberg and Wendel, 1992). However, hybrids may achieve reproductive isolation if the reproductive processes that allow backcrossing are disrupted. For example, polyploidy or chromosome recombination can lead to chromosome mispairing and associated postzygotic sterility, alternatively reproductive character displacement or hybrid superiority can lead to prezygotic reproductive isolation.

Polyploidy

Many recently evolved allopolyploids produce partially or completely sterile progeny when backcrossed to parental taxa, eg. *Tragopogon mirus* and *T. miscellus* (Ownbey, 1950; Ownbey and McCollum, 1953), *Spartina anglica* (Gray, Marshall and Raybould, 1991; Raybould *et al.*, 1991) and *Senecio cambrensis* (Ingram and Noltie, 1987). The reduction in fertility is mainly associated with uneven chromosome paring after the fusion of gametes containing different chromosome numbers (Ingram, 1977). Allopolyploidy appears to be a very successful route of producing fertile, hybrid taxa, and many allopolyploids often exhibit a number of beneficial features, besides reproductive isolation, due to the mechanisms of polyploidy and hybridization involved in their origin, e.g.: 1. Increased genetic variation following the combination of parental characters (Thompson and Lumaret, 1992) and/or multiple origins (Soltis and Soltis, 1993); 2. Possession of novel morphological and molecular variation (Rieseberg and Ellstrand, 1993; Soltis and Soltis, 1993; Soltis and Soltis, 1995; Song *et al.*, 1995); 3. Reassurance of reproductive success and true breeding, following disruption of parental self-incompatibility systems (Thompson and Lumaret, 1992).

Homoploid recombination

Postzygotic breeding barriers can arise in a homoploid hybrid by the recombination of chromosome segments that distinguish the parental species. The new recombinant types are fertile *inter se,* but at least partially sterile with both parents. The process

Table 4.1. Summary of main reproductive isolating barriers (from Levin, 1978).

Spatial	
1. Ecological	
Reproductive	
2. Temporal divergence	
(a) Seasonal	
(b) Diurnal	
3. Floral divergence	
(a) Ethological	
(b) Mechanical	
Premating	
Postmating	
4. Reproductive Mode	
5. Cross-incompatibility	
(a) Pollen-pistil	
	Prezygotic
	Postzygotic
(b) Seed	
6. Hybrid inviability or weakness	
7. Hybrid floral isolation	
8. Hybrid sterility	
9. Hybrid breakdown	

causing this phenomenon has been termed 'recombination speciation' by Grant (1981), and has been verified experimentally on a number of occasions by the resynthesis of homoploid hybrid taxa, eg. in *Gilia* (Grant, 1966) and *Nicotiana* (Smith and Daly, 1959). It is also thought that a number of natural hybrid taxa have arisen by this process, including *Stephanomeria diegensis*, *Helianthus paradoxus*, *H. anomalus*, *H. deserticola* and *Iris nelsonii* (Stebbins and Daly, 1961; Gallez and Gottlieb, 1982; Arnold, Hamrick and Bennett, 1990; Rieseberg, 1991; Rieseberg and Wendel, 1992). In the three *Helianthus* hybrids, it is known that the chromosome isolating barriers are a consequence of their hybrid origin (Rieseberg and Wendel, 1992).

Reproductive character displacement

Grant (1949) proposed that a new hybrid taxon could be reproductively isolated if it produced an intermediate flower type that was pollinated by flower-constant insects. This mode of hybrid speciation has been proposed for two species of *Penstemon* (Straw, 1955) and also for *Delphinium gypsophilum*, the hybrid derivative of *D. hesperium* and *D. recurvatum* (in Dobzansky *et al.* 1977). Expression of an intermediate or novel flower type may lead to reproductive isolation by differential attraction of selective pollinators (ethological isolation) or by the exclusion of certain promiscuous pollinators (mechanical isolation). There are only a few examples of hybrid speciation brought about by reproductive character displacement, but there are other examples where reproductive characters that isolate two parental species are displaced following hybridization, causing a change in pollinator behaviour or exclusion in hybrid progeny. Some plant families contain species that exhibit highly specialized, vector-specific flowers and are more likely to be affected by such mechanisms; for example, experimental crosses between orchid species produced hybrid progeny that exhibited a combination of parental floral traits which affected pollinator attraction (in Dobzansky *et al.*, 1977). In another study, the species specific pollinators of *Salvia mellifera* and *S. apiana*, which are maintained due to mechanical features mainly associated with flower size, were excluded from hybrid progeny following the disruption of these characters by segregation (in Solberg, 1971).

Expression of novel or intermediate characters may lead to hybrid reproductive isolation via other mechanisms. For example, a shift in the date or time of flowering following hybridization can cause seasonal or diurnal temporal divergence respectively (Levin, 1978). Studies of sympatric animal species that form hybrid zones have examined divergence of male signal or other characters affecting assortative mating as mechanisms that could reinforce mating isolation (summarized in Butlin, 1989; Howard, 1993). However, little evidence has emerged to prove convincingly that

reproductive isolation is reinforced in such hybrid zones (i.e. by selection against unfit hybrid or backcross progeny) and there are no examples of new 'hybrid' species arising by this process (Hewitt, 1988; Butlin, 1989; Butlin and Ritchie, 1994).

Disruption of flower morphology may also cause a shift of breeding system from outcrossing to autogamy which can lead to very effective reproductive isolation (Dobzansky *et al.* 1977). For example, *Epipactis youngiana*, the recently evolved, putative hybrid product of the predominantly outbreeding species, *E. helloborine* and *E. leptochila*, is thought to be mainly autogamous (Richards and Porter, 1982), although further work is required on the reproductive biology of these species to confirm this. The associated break-down of parental self-incompatibility systems in polyploid hybrids (Thompson and Lumaret, 1992), also offers great potential for reproductive isolation by autogamy.

In addition, some sterile F_1 hybrids can maintain their phenotype and prevent backcrossing to parental species through either vegetative propagation, e.g. clonal propagation in *Spartina* x *townsendii* (Gray, Marshall and Raybould, 1991) and *Potamogeton* x *schreberi* (Hollingsworth, Preston and Gornall, 1995), or apomixis in *Rosa* and the 'micro species' of *Taraxacum* (Stace, 1991). However, in evolutionary terms, asexual modes of reproduction are viewed as 'dead ends'.

Hybrid superiority

The effects of hybrid vigour are well known in plants (Stace, 1975). Whilst improving size and productivity, heterosis can manifest itself in traits such as greater resistance to disease or other environmental stresses, and has been exploited for its agronomic potential (Allard, 1960). Increases in environmental stress tolerance, could allow hybrids to colonize new habitats where parental taxa are not established, causing ecological reproductive isolation. Anderson (1948) originally postulated that F_1 hybrids between species that prefer different habitats, will exhibit an intermediate habitat requirement. However, he also deduced that each of the second generation progeny will require a particular habitat for optimal development. Anderson observed that such 'hybridized habitats' were rarely found and consequently there is a discrepancy between the high number of new hybrids formed and their relatively low frequency of establishment. Since Anderson, two main models have emerged to predict the fitness of hybrids in natural populations. The bounded hybrid superiority hypothesis, predicts that hybrid fitness will be superior to parental populations only in certain habitats (Moore, 1977). In contrast, the dynamic equilibrium model, predicts that hybrids will exhibit lower fitness than parental taxa regardless of habitat (Barton

and Hewitt, 1985). A recent review showed that in many cases hybrid fitness was equal to or greater than parental taxa in at least some habitats (Arnold and Hodges, 1995), favouring the bounded hybrid superiority hypothesis. Notable examples of superior hybrid fitness include increased temperature tolerance exhibited by hybrids between *Dacus tryoni* and *D. neohumeralis* (Lewontin and Birch, 1966), and the occurrence of certain *Iris fulva* x *I. brevicaulis* hybrids in semi-aquatic habitats, unlike the habitats where parental species are found (Arnold and Hodges, 1995). Abbott (1992) noted that the successful establishment of hybrids often follows the colonization of a new habitat eg. *Lantana camara* and *Spartina anglica* (Thompson, 1991), and may be a more general feature of successful hybrid speciation events.

Reproductive isolation of fertile *Senecio* hybrids and derivatives

The hexaploid *S. cambrensis*, maintains a postzygotic breeding barrier with respect to its parental taxa due to its allopolyploid origin. Backcrosses to both *S. vulgaris* and *S. squalidus* produce offspring with reduced fertility (Ingram and Noltie, 1995). This postzygotic breeding barrier seems strong enough to isolate *S. cambrensis*, and backcross progeny have never been reported from the wild (Ingram and Noltie, 1995).

It is not expected that reproductive barriers are likely to occur to the same extent between the introgressant *S. vulgaris* var. *hibernicus* and its parental taxon *S. vulgaris* var. *vulgaris* (Abbott, 1992). *S. vulgaris* var. *hibernicus* is only found in nature in mixed stands with var. *vulgaris*, and the two taxa are easily crossed to produce fertile progeny (Trow, 1912). The level of crossing between var. *hibernicus* and var. *vulgaris* can be high (up to 35% in some populations, Marshall and Abbott, 1984a) and consequently no genetic differentiation between the two morphs is expected, except those associated with the ray floret locus. However, the two variants have been shown to differ at an allozyme locus that was unlinked to the ray floret locus (Abbott, Ashton and Forbes, 1992), and in several life history characters, including; germination behaviour (Richards, 1975; Abbott, 1986; Abbott, Horril and Noble, 1988), seedling survivorship and fecundity (Abbott and Horril, 1991), speed of development (Richards, 1975; Kadereit and Briggs, 1985; Abbott, 1986; Oxford, Crawford and Perneys, 1996) and sometimes reproductive fitness (Oxford and Andrews, 1977). These observations lead Abbott, Irwin and Ashton (1992) to propose that a 'coadapted' complex of genes may have been introgressed into *S. vulgaris* from *S. squalidus*, and may be currently maintained in var. *hibernicus* due to the reduced fitness of backcross progeny (Abbott, 1992).

The breeding system of the two varieties of *S. vulgaris* has been the subject of considerable research. *S. vulgaris* var. *vulgaris* is a predominant selfer (Gibbs, Milne and Vargas-Carillo, 1975; Marshall and Abbott, 1982) possessing characters associated with a well-established inbreeder, including, inconspicuous capitula, low pollen yield and a high recombination index. *S. vulgaris* var. *hibernicus* is mainly distinguished from var. *vulgaris* by the presence of an outer whorl of approximately 8-13 ray florets in its capitula. The ray florets are male sterile and have been inherited from *S. squalidus* following introgression into *S. vulgaris* (Ingram, Weir and Abbott, 1980; Ingram and Taylor, 1982), and make the capitula of var. *hibernicus* more showy and potentially more attractive to pollinators than var. *vulgaris*.

Estimates of intermorph outcrossing vary, and Trow (1912) found that levels were usually around 1% but could rise to 10%. Hull (1974a) reported that in Scotland outcrossing between the two morphs rarely exceeded 1%, although could go up to 15% in some populations. However, Campbell and Abbott (1976) found that intermorph outcrossing reached 22.4% when non-radiate plants were closely surrounded (6 cm) by radiate plants. Marshall and Abbott (1982, 1984a, b) have shown that radiate plants often exhibit considerably higher levels of female outcrossing (35% at peak periods of outcrossing) relative to non-radiate plants (normally less than 1% at all times of the year) in mixed populations of the two variants.

Further examination of outcrossing frequency of ray and disc florets of radiate plants in one population, revealed that the higher frequency of outcrossing in radiate plants was entirely due to higher outcrossing rate of the pistillate ray florets relative to the hermaphrodite disc florets (Marshall and Abbott, 1984b). However, in another population, the higher outcrossing rate of radiate plants was only partially explained by increased outcrossing in ray florets (Marshall and Abbott, 1984b). Marshall and Abbott (1984b) speculated that in this population the higher outcrossing of radiate plants might stem from differences in pollinator movement between morphs. To examine this further, Abbott and Irwin (1988) studied pollinator preference in experimental plots containing equal numbers of radiate and non-radiate plants. Syrphid flies were found to visit radiate capitula preferentially to non-radiate capitula in these stands, at a ratio of 0.62 to 0.38. This preference was expressed initially when pollinators entered plots and was maintained throughout the period that a pollinator foraged in a plot. One effect of this preference is to transfer pollen from non-radiate to radiate plants more frequently than in the opposite direction, causing radiate plants to exhibit a higher level of intermorph outcrossing. Another effect is to cause the radiate morph to exhibit greater intramorph outcrossing than the non-radiate morph.

Both of these predictions were confirmed by Irwin (1990) in studies of intra- and intermorph outcrossing rates that employed marker genes in experimental plots of radiate and non-radiate plants.

Irwin (1990) identified a sample of the pollinators observed in her experimental plots and found that they were all common species of syrphid flies that occur in Scotland. Hoverflies feed on nectar, and pregnant females seek the proteins and amino acids in pollen for egg provision. Young males also require the proteins from pollen for genital maturation (Gilbert, 1986). Thus the pollen and functional nectaries in groundsel are both important food sources to its main pollinators. Hoverflies can identify mates up to one metre away (Stubbs and Falk, 1983), and presumably would be able to see flowering stands at a greater distance, making them potentially important agents in the selective transfer of groundsel pollen in natural populations.

In addition to Abbott's (1992) proposal that progeny of intermorph crosses may experience a postzygotic reduction in fitness, the above studies show that there is also a degree of prezygotic reproductive isolation between the two morphs of groundsel; firstly due to the predominant autogamy exhibited by both morphs (although lower in the radiate morph), and secondly due to the behaviour of its main pollinators, which preferentially select radiate over non-radiate capitula, thus reducing the overall level of intermorph crossing.

Reproductive isolation of York radiate groundsel from its parental taxa

York radiate groundsel has maintained its distinctive hybrid morphological phenotype in populations around York since its discovery in 1979 (Chapter 2; Irwin and Abbott, 1992), and in addition, has maintained a distinctive isozyme profile since at least 1991 (Chapter 2; Chapter 3; Irwin and Abbott, 1992). Backcrossing to *S. vulgaris* or *S. squalidus* would be expected to have disrupted both of these phenotypes (as was shown in a morphological examination of backcross progeny in Chapter 3) and this together with the fact that very few (if any) backcross products are seen in the field, suggests that York radiate groundsel is effectively reproductively isolated from its parental taxa. An examination of how such isolation is achieved should provide exciting insights into the evolution of isolating mechanisms in hybrid derivatives such as York radiate groundsel.

In Chapter 3, crosses made between York radiate groundsel and *S. squalidus* produced highly sterile progeny and demonstrated that there was a postzygotic chromosomal breeding barrier in place between these two taxa caused by the

difference in ploidy level. Observations of field populations have revealed that *S. squalidus* rarely co-occurs with York radiate groundsel at the main sites in York. On the rare occasions that the two taxa do occur sympatrically, postzygotic reproductive isolation would be enforced by the existing chromosome barrier. Consequently further possible isolating mechanisms between the two taxa are not considered here.

S. vulgaris var. *vulgaris* and York radiate groundsel are both tetraploid and occur sympatrically at most sites in York and so there exists considerable potential for inter-crossing to occur.

Objectives

In the work reported in this chapter, postzygotic breeding barriers between York radiate groundsel and *S. vulgaris* var. *vulgaris* were investigated by examining the fertility of first and second generation progeny of crosses between the two taxa. In addition, the existence of prezygotic breeding barriers were examined taking into account differences in flowering time and their causes, e.g. differences in germination behaviour over a range of temperatures, speed of development, and seedling establishment and survival in the wild over winter. Also the level of intertaxon crossing was assessed in the field along with an examination of how pollinator behaviour affects intertaxon crossing level.

Methods

Experimental crosses between York radiate groundsel and *S. vulgaris*

Reciprocal crosses were made between selected individuals of York radiate groundsel from Lendal Bridge and *S. vulgaris* var. *vulgaris* from Methil, as described in Chapter 3, and F_1, F_2 and reciprocal backcross generations were synthesized. From the entire set of parental and hybrid progeny cultivated as described in Chapter 3, three fertility and fitness characters were measured on 209 individuals, including, 33 plants of *S. vulgaris* var. *vulgaris*, five plants of var. *hibernicus*, 40 plants of York radiate groundsel, 18 F_1 hybrids, 72 F_2 hybrids and 41 reciprocal backcrosses. Pollen fertility was assessed by examining the proportion of grains stained with acetocarmine, while seed fertility was assessed by determining the proportion of seeds that matured in a capitulum left to open-pollinate in a glasshouse or forced to self. If the two values for seed set were similar they were averaged; if not, the open seed set proportion was used in analysis. Life time production of capitula was also estimated after leaving plants to grow under glass until natural death (up to a year). These measures of fitness and fertility were subjected to one-way ANOVA, and significant differences between taxa and generations were assessed by means of Tukey-Kramer multiple comparisons. A single F_2 plant that exhibited low fertility was also subjected to examination of meiotic chromosome pairing behaviour.

Observation of flowering time

In 1993, the total number of flowering individuals of *S. vulgaris*, York radiate groundsel and *S. squalidus* were recorded from seven sites around York (see Figure 3.4; site 1, Dalton Terrace; site 2, NW Lendal Bridge; site 3, SW Lendal Bridge; site 4, SE Lendal Bridge; site 6, Victoria House car park; site 7, All Saints Church; site 9, Spotted Cow pub). Records were taken on most or all of four sample dates (6.5.93, 10.6.93, 30.6.93 and 30.9.93). In 1994, the total number of flowering individuals and the total number of flowering capitula per plant were recorded for the same three taxa at eight sites around York (see Figure 3.4; sites 1, 2, 3, 4, 6, 9, including site 5, Lendal Bridge park and site 8, Viking Hotel) on most or all of seven sample dates (23.3.94, 21.4.94, 18.5.94, 7.6.94, 14.7.94, 5.8.94 and 22.9.94).

Germination behaviour

Fresh seed (not less than two weeks old) of *S. vulgaris* var. *vulgaris*, var. *hibernicus*, York radiate groundsel and *S. squalidus* was left to germinate at five temperatures (5, 10, 15, 20 and 25° C) for 45 days (from 27.4.94). Each temperature/taxon treatment was replicated 12 times and consisted of 20 seeds placed on strips of moist 3MM blotting paper. The blotting paper strips were supported by plastic beads floating in

the well of a thermogradient bar filled with water. A detailed description of the design and dimensions of this thermogradient bar is given by Horril (1989). Seeds were examined every day, and those that had germinated were removed. Results for analysis were expressed for each day as the cumulative proportion of seeds germinated out of 20. Data were arcsine transformed before ANOVA.

Seedling establishment at Dalton Terrace, York

On 22.9.94 it was discovered that the Dalton Terrace site had been completely weeded and cleared. Advantage was taken of this event to investigate subsequent seedling establishment and survival at the site. *S. vulgaris* and York radiate groundsel seedlings are easily distinguished at the first true leaf stage and so it was possible to classify seedlings according to type. Eight one metre long linear transects were marked out along the edge of the site and visited on 26.11.94, 16.2.95, 5.3.95 and 25.7.95. Over this period a record was taken of the number of individuals of each taxon present (established and new), the total number of leaves per plant (first three dates only), and the number of flowering capitula per plant (last two dates only).

Intertaxon crossing in the wild

Samples of field pollinated seed from *S. vulgaris*, York radiate groundsel and some *S. squalidus* plants were collected from most flowering individuals at six sites (sites 1, 2, 3, 4, 7 and 9) on two dates (6.5.93 and 30.6.93) in 1993, and at seven sites (sites 1, 2, 3, 4, 5, 8 and 9) on four dates (21.4.94, 18.5.94, 7.6.94 and 22.9.94) in 1994. Where possible, ten seeds were sampled from each individual per site per date and offspring produced from the seeds were grown under glass until flowering. Hybrids between York radiate groundsel and *S. vulgaris* in the offspring of these two taxa were easily distinguished by the possession of short stubby ray florets and an intermediate leaf morphology.

Data from the population at Dalton Terrace in 1993 was used to estimate the frequency of the radiate allele in the population at this site, Wright's fixation index and the outcrossing rates of *S. vulgaris* and York radiate groundsel following the procedures described in Marshall and Abbott (1982).

The frequency of the radiate allele was calculated from the population genotype frequencies at the ray floret locus using the equation:

$$\text{Frequency of the radiate allele,} \qquad Tr = \frac{2a + h}{2N}$$

Where a is the number of radiate plants in the population, h is the number of heterozygotes and N is the total number of individuals in the population. The variance of gene frequency is given by:

$$\text{var. } (Tr) = \frac{Tr \,.\, Tn\,(1+F)}{2N}$$

Where *Tn* is the frequency of the non-radiate allele (*Tn* = 1 - *Tr*), and F is Wright's fixation index (see below).

Wright's Fixation Index is estimated from the population census data. It is a measure of departure from the genotypic proportions expected under panmixia. The maximum likelihood estimate of the Fixation Index (F) for the ray floret locus is estimated as:

$$F = \frac{4a \,.\, b\text{-}h^2}{(2a+h)(2b+h)}$$

with variance:

$$\text{var } (F) = \frac{(1-F)\{2Tr \,.\, Tn + 2\,(1-3Tr \,.\, Tn)F - (1 - 4Tr \,.\, Tn)F^2\}}{2NTr \,.\, Tn}$$

Where b is the number of non-radiate plants in the original population. High levels for F are expected with inbreeding and are associated with low levels of heterozygosity.

The maternal outcrossing rates of York radiate groundsel (*TrTr*) and *S. vulgaris* var. *vulgaris* (*TnTn*) genotypes were estimated using the following equations:

$$t_{(TrTr)} = \frac{H_{(TrTr)}}{Tn}$$

$$t_{(TnTn)} = \frac{H_{(TnTn)}}{Tr}$$

Where $H_{(TrTr)}$ and $H_{(TnTn)}$ are the frequencies of heterozygotes in the progenies of radiate and non-radiate homozygotes respectively and t equals outcrossing rate.

The variance of these estimates is derived from the equation:

$$\text{var}\,(t_{(TrTr)}) = \frac{1}{Tn} \cdot \frac{H_{(TrTr)}\ (1 - H_{(TrTr)})}{N} + \frac{(H_{(TrTr)})^2}{Tn^2} \cdot \frac{Tn(1 - Tn)}{Np}$$

Where Np is the number of progeny scored in the estimate of heterozygote frequency. The appropriate substitutions are made to calculate the variance of non-radiate plants.

Intertaxon crossing under experimental conditions

Taxon lines had previously been pure bred for allozyme phenotype at the *Aat-3* locus, such that York radiate groundsel exhibited the *b* phenotype, *S. vulgaris* (var. *vulgaris* and var. *hibernicus*) plants the *a* phenotype and *S. squalidus* plants were homozygous for the *c* allele. Seeds of *S. squalidus*, York radiate groundsel and *S. vulgaris* var. *vulgaris* and var. *hibernicus* from the pure bred lines were sown in 7 cm pots. Sowing was staggered to ensure that plants flowered synchronously. Just before flowering, all plants were transplanted to six different planting designs in the St Andrews Botanic Garden. In three planting designs, York radiate groundsel plants were surrounded by either *S. vulgaris* var. *vulgaris*, var. *hibernicus* or *S. squalidus*, while in the other three designs York radiate groundsel individuals surrounded central plants of either *S. vulgaris* var. *vulgaris*, var. *hibernicus* or *S. squalidus*. Each of the six planting designs was repeated for three sizes of plot. The smallest plot had one 'receiver' plant in the centre of the plot surrounded by four 'donor' plants placed 15 cm from the central plant. The medium sized plot comprised two of the smallest plot designs set side by side (while keeping 15 cm between the outer plants of duplicated plots) and the largest plot comprised four of the smallest plot designs arranged in a square array. For every plot, three recently opened capitula were selected on the central plant(s), and were tagged and left to open pollinate over a three day period, before being enclosed in a bag. Seed was collected in this way from capitula during September 1993 on six sample dates. Seed set in *S. squalidus* plants that were surrounded by York radiate groundsel plants was very low and consequently progeny were not analysed. A random sample of up to 20 seed from each capitulum was sown out in 15 cm pots. In total, 3899 seed from 270 capitula were sown out, and progeny were raised to flowering under glass to examine the frequency of intertaxon outcrossing. Hybrid plants were identified by morphology, and confirmed by isozyme analysis. For analysis the number of intertaxon hybrids was expressed as a proportion of the total number of seed planted out per capitulum (up to 20).

Results

Fertility of F_2 and backcross offspring

No significant difference was found in the proportion of seed set between conditions of open or self-pollination in York radiate groundsel, *S. vulgaris* var. *vulgaris* or var. *hibernicus* or any of the F_1, F_2 or B_1 hybrid progeny and so all seed set values are a mean of both pollination conditions. Male and female fertility in F_2 progeny was significantly reduced relative to the fertility of York radiate groundsel and *S. vulgaris* var. *vulgaris* parental lines (Table 4.2). Mean seed set in the F_2 was 58.8%, however, variation was great and some individuals set no seed while others exhibited 89% seed set. Backcross progeny showed a return to high seed fertility which was not significantly different in mean from that exhibited by York radiate groundsel; however, B_1 progeny again exhibited a wide range of fertility and were not significantly different in mean fertility from F_2 progeny. For pollen fertility, F_2 progeny also exhibited a significantly lower mean than *S. vulgaris* var. *vulgaris* and York radiate groundsel plants (range 62% to 100%), but were similar in this regard to B_1 progeny derived from crosses to *S. vulgaris* var. *vulgaris*; however, B_1 progeny derived from crosses to York radiate groundsel, showed a significant increase in fertility relative to F_2 progeny and expressed a mean not significantly different from that of the parental taxa. No significant difference in mean lifetime production of capitula was found between York radiate groundsel or *S. vulgaris* var. *vulgaris* or their F_1, F_2 or B_1 hybrid progeny.

Meiotic chromosome preparations of a partially sterile F_2 plant produced from the cross showed that it was tetraploid, but produced on average two univalents indicating some chromosome mispairing.

Flowering time

Most sites in York were subject to considerable disturbance (weeding, herbicide treatment and drought) during the main summer period. This made flowering time comparisons between taxa difficult (see Table 4.3). However, the data for the Dalton Terrace site were most complete, and a plot of the number of plants flowering at each sample date showed that York radiate groundsel was later to flower in both 1993 and 1994 (Figure 4.1 and 4.2). This difference is made even more evident from the total number of flowering capitula recorded on plants at Dalton Terrace in 1994 (Figure 4.3). It was of interest that two York radiate groundsel x *S. vulgaris* hybrids were recorded at Dalton Terrace in 1993. However, no such hybrids were found at the site in 1994 nor at any other site surveyed in 1993 and 1994.

Table 4.2. Means, standard deviations, significant differences (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns-not significant) and results of Tukey-Kramer multiple comparison for three fertility or fitness traits measured on York radiate groundsel and *S. vulgaris* var. *vulgaris* parental, F_1, F_2 and reciprocal backcross lines. Five individuals of *S. vulgaris* var. *hibernicus*, grown at the same time, were included for comparison. Means sharing the same superscript are not significantly different ($P \leq 0.05$). Standard deviations are in parentheses.

Taxa	*S. vulgaris* v. *vulgaris*	*S. vulgaris* v. *hibernicus*	York radiate groundsel	F_1	F_2	B_1 to *S. vulgaris* var. *vulgaris*	B_1 to York radiate groundsel	
Character								*P*
N=	33	5	40	18	72	21	20	
Pollen fertility %	97.6^{b} (3.2)	97.2^{abc}(3.7)	99.6^{a} (0.5)	97.0^{bc}(3.4)	94.5^{cd}(7.5)	92.1^{d} (8.1)	98.4^{ab}(2.5)	***
Seed set %	82.4^{a}(12.4)	82.4^{abc}(10.9)	78.5^{ab} (7.9)	63.8^{bc}(16.6)	58.8^{c} (26.5)	66.1^{abc}(36.6)	67.2^{bc}(18.2)	***
N=	19	5	11	10	40	18	10	
Total lifetime production of capitula	80.4^{b}(26.4)	120.6^{a}(15.9)	102.2^{ab}(22.8)	96.9^{ab}(35.7)	87.6^{b}(32.1)	78.9^{b}(30.1)	78.8^{b}(18.9)	*

Table 4.3. Number of plants of each taxon flowering at a particular site on a specific day in 1993. Records include notes on site disturbance.

Date Site	6.5.93	10.6.93	30.6.93		30.9.93
Spotted cow					
S. vulgaris var. *vulgaris*	?	-	25		?
S. vulgaris with York leaves	?	25	17		?
Barbican					
S. vulgaris with York leaves	?	?	2		?
Leicester way					
S. vulgaris with York leaves	?	?	1		?
All Saints church					
York radiate groundsel	?	?	1		?
S. squalidus	?	?	1		?
Victoria House car park					
S. vulgaris var. *vulgaris*	?	20	14		?
York radiate groundsel	?	1	3		?
Ouse Bridge					
S. vulgaris var. *vulgaris*	8	?	weeded		
S. squalidus	1	?	weeded		
Lendal Bridge SE					
York radiate groundsel	-	-	-	site	1
S. squalidus	-	-	-	flooded	3
				(16.9.93)	
Lendal Bridge SW					
S. vulgaris var. *vulgaris*	6	?	0	site	
York radiate groundsel	1	?	1	cleared	3
S. squalidus	12	?	>200		31
Lendal Bridge NW					
S. vulgaris var. *vulgaris*	31	?	0	site	
S. vulgaris var. *hibernicus*	11	?	1	flooded	
S. squalidus	7	?	55	(16.9.93)	9
Dalton Terrace					
S. vulgaris var. *vulgaris*	57	13	4	site weeded	20
York radiate groundsel	36	56	40	and cleared	8
Heterozygote	2	1	0		

Table 4.3. Continued for 1994.

Date Site	23.3.94	21.4.94	18.5.94	7.6.94	14.7.94	5.8.94	22.9.94
Spotted cow							
No. *S. vulgaris* var. *vulgaris*	3	13	weeded	10	6	?	20
No. *S. vulgaris* with York leaves	0	0	weeded	7	12	?	0
Viking Hotel							
No. York radiate groundsel	0	0	1	1	weeded		
Victoria House car park							
No. *S. vulgaris* var. *vulgaris*	0	10	10	10	weeded		
No. *S. squalidus*	0	1	1	1	weeded		
Lendal Bridge NW							
No. *S. vulgaris* var. *vulgaris*	6	9	19	3	herbicide	cleared	2
No. *S. vulgaris* var. *hibernicus*	1	dead			treated		
Lendal Bridge SW							
No. *S. vulgaris* var. *vulgaris*	13	23	herbicide			cleared	11
No. York radiate groundsel	1	3	treated				0
No. *S. squalidus*	0	83					5
Lendal Bridge SE							
No. *S. vulgaris* var. *vulgaris*	0	0	1	severe		4	8
No. York radiate groundsel	3	3	5	drought		9	17
No. *S. squalidus*	1	weeded	13	all dead	1	9	0
Lendal Bridge park							
No. *S. vulgaris* var. *vulgaris*	5	1(weeded)	10	weeded		5	19
No. York radiate groundsel	1	1	1	1	1	1	2
Dalton Terrace							
No. *S. vulgaris* var. *vulgaris*	17	32	21	17	1	dead	weeded
No. York radiate groundsel	2	6	11	12	2	dead	cleared

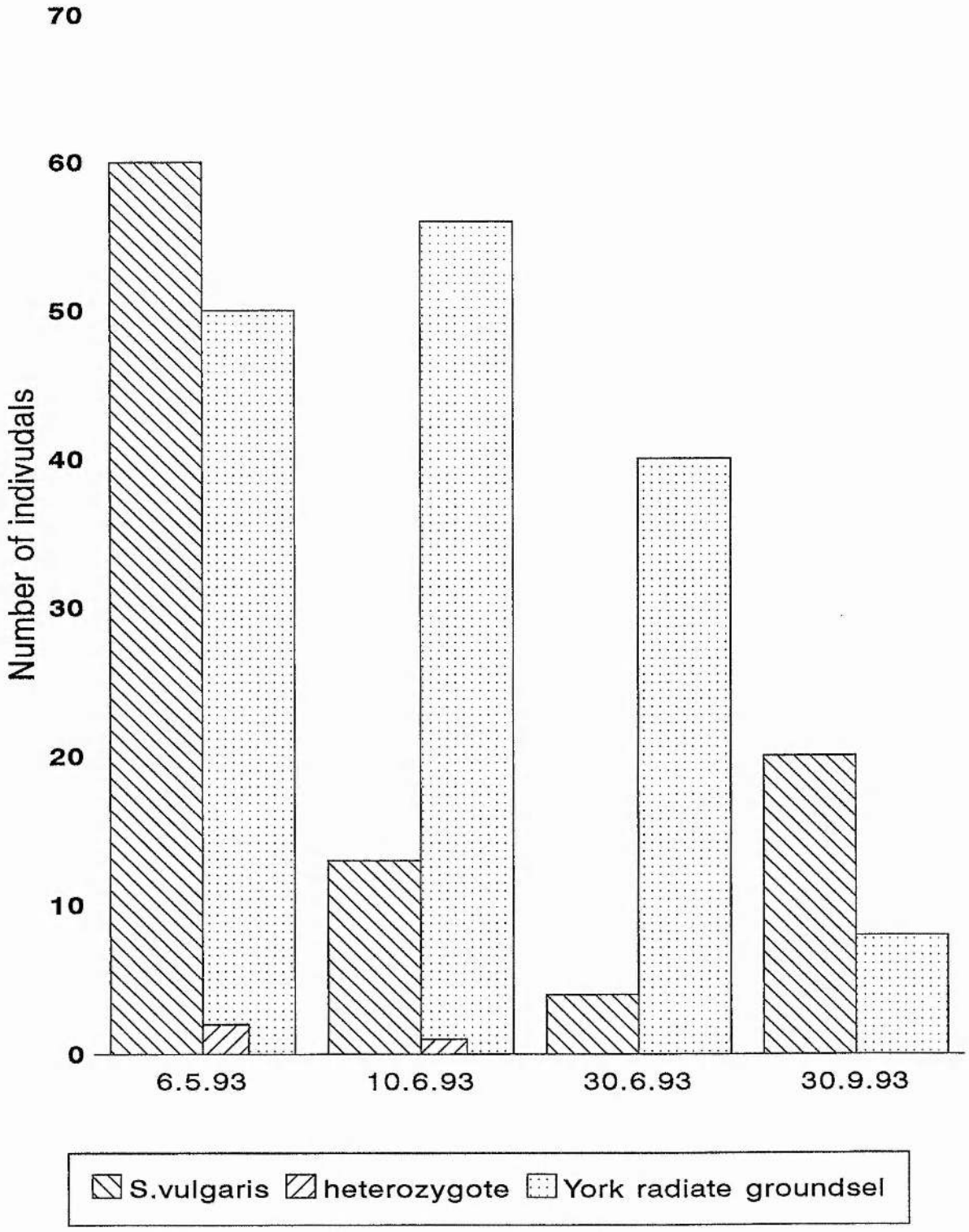


Figure 4.1. Numer of individuals of each taxon flowering on a specific date at Dalton Terrace during 1993. Heterozygote refers to York radiate groundsel x *S. vulgaris* hybrid.

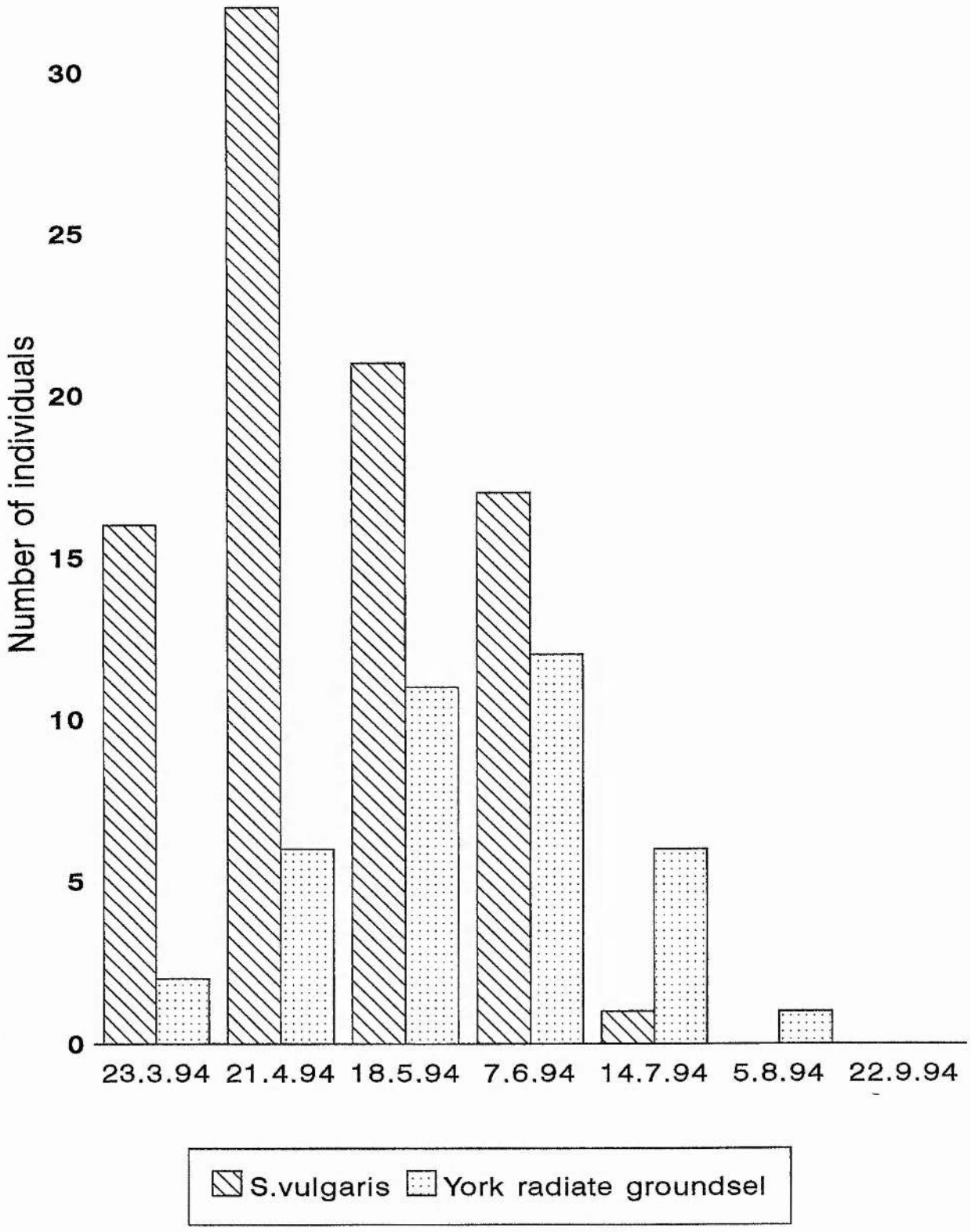


Figure 4.2. Number of individuals of each taxon flowering on a specific date at Dalton Terrace during 1994.

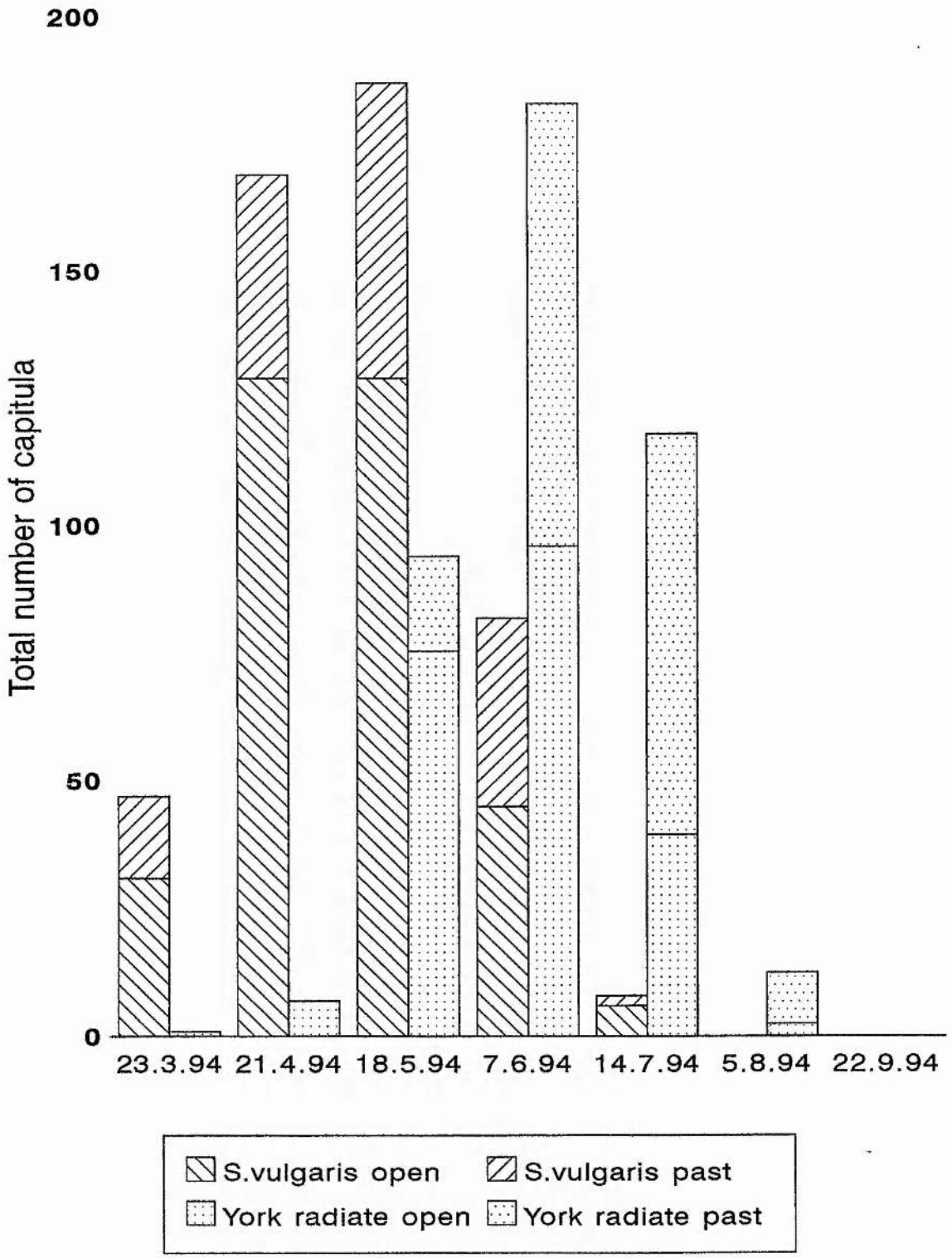


Figure 4.3. Total number of flowering capitula produced by all plants of each taxon on a specific date at Dalton Terrace during 1994. *Open*, refers to capitula that were anthesing on the sample date, *past*, refers to those capitula that had finished anthesing but before achenes were mature and shed from the capitulum.

Seed germination

Seeds of York radiate groundsel germinated earlier than seeds of *S. vulgaris* var. *vulgaris* at low temperatures (5 to 15° C, Figure 4.4a, b and c), while at 20° C there was no difference (Figure 4.4d), and at 25° C the reverse was true (Figure 4.4e). Seeds of York radiate groundsel showed a similar pattern of germination to those of *S. squalidus* between 10 to 20° C, but at 5° C were earlier to germinate and at 25° C were later to germinate. *S. vulgaris* var. *hibernicus* seed was typically slow to germinate at all temperatures.

Seedling establishment and survival

Based on observations from the eight linear transects at Dalton Terrace, made between November 1994 and August 1995, it was evident that many more *S. vulgaris* than York radiate groundsel seedlings were present at the site in November 1994. However, by February 1995, this difference was much reduced (Figure 4.5). The number of individuals of both taxa continued to decline at the site until July 1995, with very few new seedlings of either taxon becoming established after winter. York radiate groundsel and *S. vulgaris* seedlings had similar numbers of leaves per individual in November 1994 (Figure 4.6); however, after winter (February and March), significantly ($P < 0.05$) more leaves were present on *S. vulgaris* than York radiate groundsel individuals. It was also evident from the number of capitula recorded per plant in March and July, 1995, that *S. vulgaris* individuals again flowered earlier than York radiate groundsel plants (Figure 4.6).

The success of *S. vulgaris* individuals to over-winter seemed to be correlated with the number of leaves they had produced before winter. Those *S. vulgaris* plants that successfully over-wintered (still alive in February) had significantly more leaves on 26.11.94 (T-test, $P < 0.001$; mean number leaves = 7.53, 40 plants) relative to those plants that had died (mean number of leaves = 5.94, 49 plants). This was not evident for York radiate groundsel individuals (T-test, not significant; mean number of leaves on plants that survived wintered = 6.86, 18 plants; mean number of leaves on plants that died during winter = 6.94, seven plants).

Intercrossing between York radiate groundsel and *S. vulgaris* in the wild

The frequency of the radiate allele and the rate of intertaxon outcrossing in the population at Dalton Terrace during 1993 are presented in Table 4.4. The frequency of the radiate allele increased markedly over the season (0.389-0.909) as expected from the difference in flowering time between York radiate groundsel and *S. vulgaris* var. *vulgaris* reported at this site (Figure 4.1). The high values for Wright's fixation

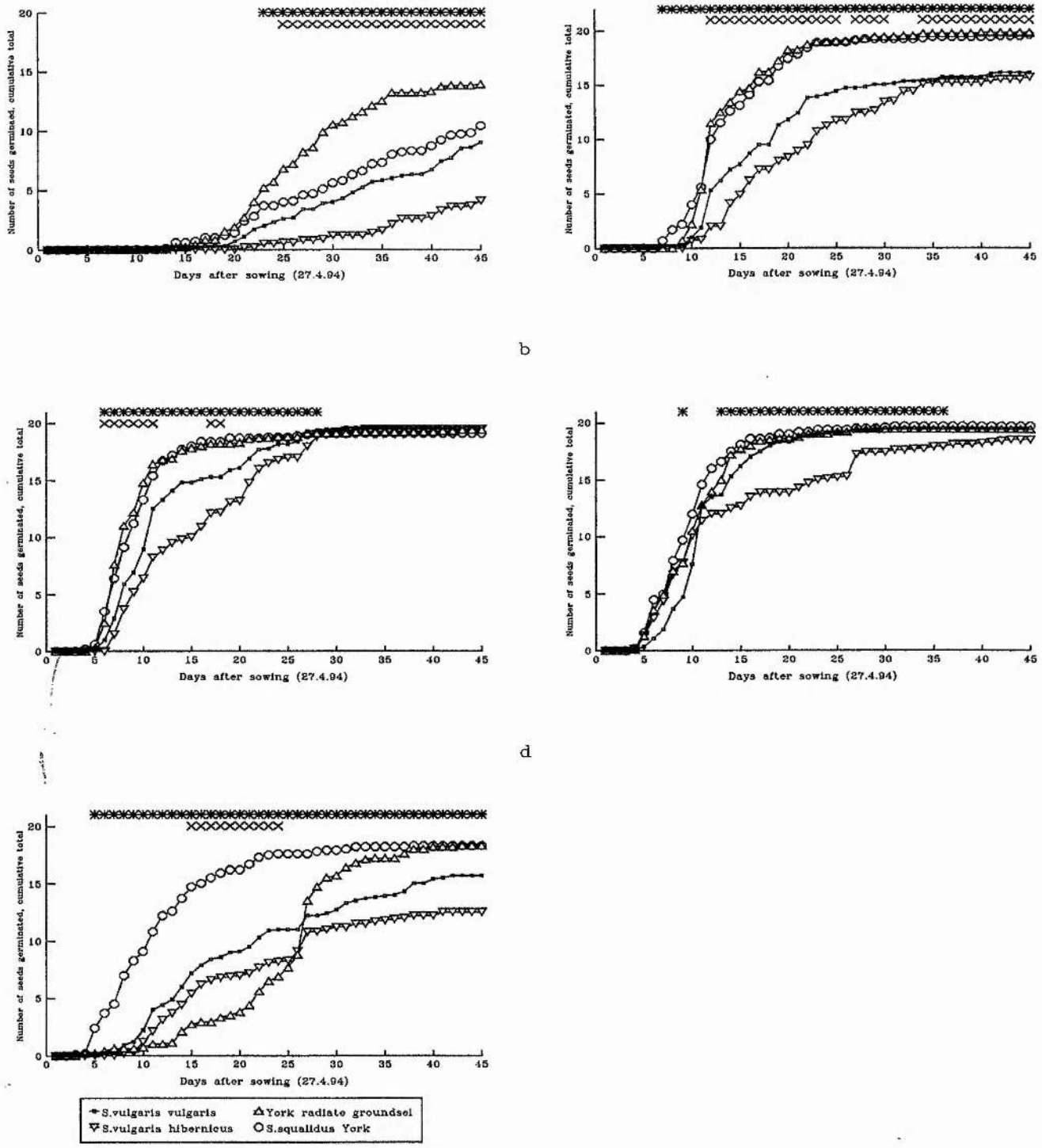


Figure 4.4. Cumulative total number of seeds germinated over time for specified taxa over a range of temperatures. Temperature treatments were; a, 5° C; b, 10° C; c, 15° C; d, 20° C; e, 25° C. Proportion of seeds germinated on each day were arcsine transformed and subjected to ANOVA, asterisks indicate that there was a significant difference between at least two of the taxa, crosses indicated that there was a significant difference ($P < 0.05$) in the proportion of seeds germinated between *S. vulgaris* var. *vulgaris* and York radiate groundsel.

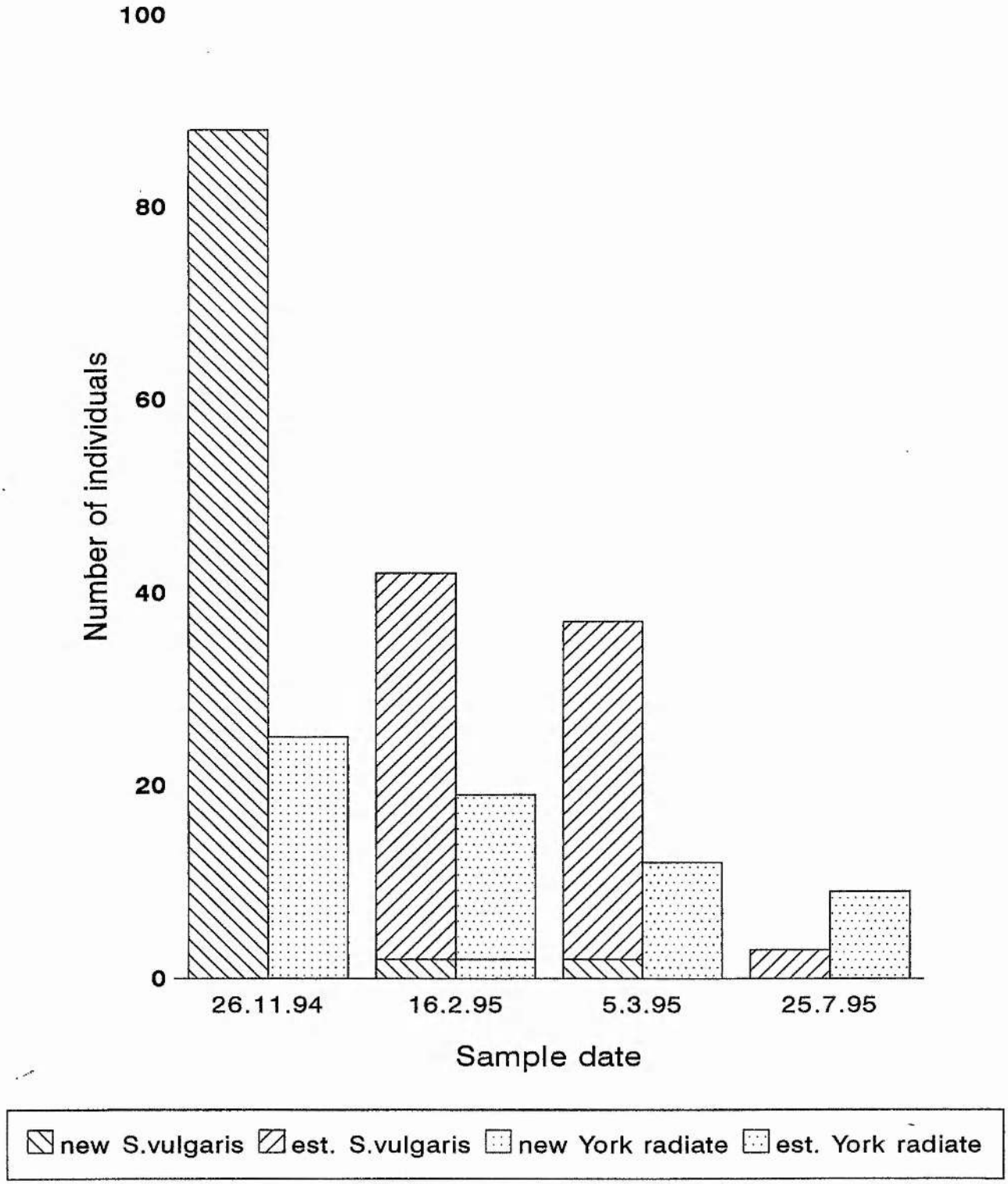


Figure 4.5. Total number of new and established (est.) seedlings of each taxon recorded on specific dates at Dalton Terrace.

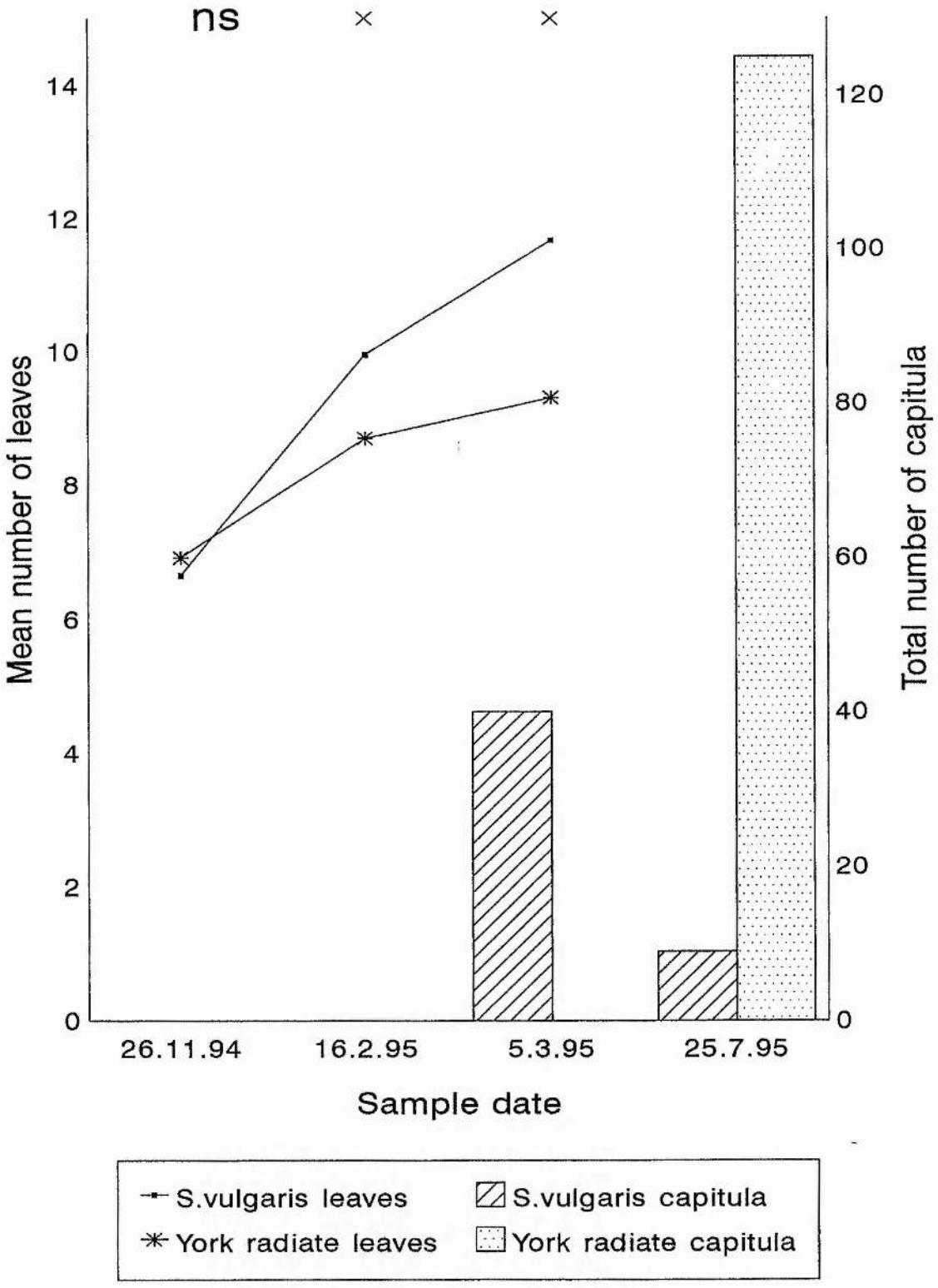


Figure 4.6. Mean number of leaves per plant and total number of flowering capitula produced by all plants of each taxon on specific dates at Dalton Terrace. Crosses indicate a significant difference ($P < 0.05$) in the mean number of leaves per plant between taxa, ns is not significant.

Table 4.4. Number of plants of each taxon present in population, number of hybrid over total progeny scored (with number of maternal plants shown in parentheses), parental Genotype frequencies (*TrTr*: *TrTn*: *TnTn*), Wright's Fixation Index (F), the frequency of the radiate allele (*Tr*) and the maternal outcrossing frequencies t at Dalton Terrace in 1993. Standard deviations are given in parentheses for F, *Tr* and $t_{(Tr,Tr)}$.

Date	6.5.93	10.6.93	30.6.93	30.9.93
Field plants				
York radiate groundsel	36	56	40	8
S. vulgaris var. *vulgaris*	57	13	4	20
Heterozygote	2	1	0	0
Hybrid and total progeny scored				
York radiate groundsel	2/223 (36)	1/475 (50)	0/268 (34)	-
S. vulgaris var. *vulgaris*	0/396 (57)	0/127 (9)	0/19 (3)	-
TnTn	0.600	0.186	0.091	0.714
TrTn	0.021	0.014	0	0
TrTr	0.379	0.800	0.909	0.286
Tr (0.007)	0.389 (0.239)	0.807 (0.535)	0.909 (0.406)	0.286
F	0.956 (0.138)	0.954 (0.080)	1.000	1.000
$t_{(TrTr)}$	0.0146 (0.009)	0.0109 (0.012)	0	-
$t_{(TnTn)}$	0	0	0	-

Table 4.5. Percentage of progeny produced by intertaxon outcrossing, recorded in a garden experiment for particular planting designs. Means sharing the same superscript are not significantly different ($P \leq 0.05$), and standard deviations are shown in parentheses. Data from plots of different sizes (small, medium and large) and from the six different sample dates were combined for each planting design. For each planting design, a total of 54 capitula were sampled and outcrossing was estimated on progeny raised from of up to 20 seeds sampled per capitulum, the total number of seeds sampled is indicated.

Central plant	York radiate groundsel	York radiate groundsel	York radiate groundsel	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *hibernicus*	
Surround plants	*S. squalidus*	*S. vulgaris* var. *vulgaris*	*S. vulgaris* var. *hibernicus*	York radiate groundsel	York radiate groundsel	*P*
No. progeny tested	769	703	887	713	827	
Percentage of progeny produced by intertaxon outcrossing	0.09 (0.09)^{c}	18.27 (2.40)^{a}	10.18 (1.48)^{b}	1.45 (0.72)^{c}	2.80 (0.76)^{c}	***

index ($\geq$ 0.95) indicate that levels of heterozygosity at the ray floret locus were low, as expected for predominantly selfing plants. The levels of female intertaxon outcrossing were extremely low for York radiate plants (0.0000-0.0146), while *S. vulgaris* var. *vulgaris* showed no female intertaxon outcrossing.

There was no evidence of intertaxon outcrossing occurring at Dalton Terrace in 1994 (no hybrids recorded among 797 progeny from 57 *S. vulgaris* plants or 200 progeny from 17 York radiate groundsel plants collected over three dates), nor at Lendal Bridge in 1993 (314 progeny tested from 47 *S. vulgaris* plants, 91 progeny from 15 *S. squalidus* plants and 53 progeny of 11 plants of York radiate groundsel collected over two dates) nor in 1994 (542 progeny tested from 40 plants of *S. vulgaris* and 197 progeny from 25 York radiate groundsel collected over four dates).

Experimental measure of outcrossing at a garden site

Estimates of intertaxon outcrossing made in the experimental garden plot were combined for the six sample periods, and over the three plot sizes (Table 4.5). The highest mean level of intertaxon outcrossing was observed for plots in which York radiate groundsel was surrounded by *S. vulgaris* var. *vulgaris* individuals (mean = 18.3%); the mean level of intertaxon outcrossing in the reciprocal planting design was only 1.45%. The next highest level of intertaxon crossing was observed in plots in which York radiate groundsel was surrounded by *S. vulgaris* var. *hibernicus* individuals (mean = 10.18%). In contrast, the mean level of intertaxon crossing in the reciprocal planting design was only (2.80%). One triploid F_1 hybrid was raised from seed collected from plots in which York radiate groundsel was surrounded by *S. squalidus* plants and consequently the mean level of intertaxon crossing here was very low (0.09%). The fertility of this hybrid, and its progeny were analysed in Chapter 3.

Discussion

The discovery of a true breeding hybrid soon after its origin offers the opportunity to study the presence of breeding barriers that may isolate it from its parent taxa. Through such study it should be possible to assess the role of hybridization in the origin of breeding barriers and the selection pressure that may maintain them. Such an opportunity was presented following the discovery of the recently originated York radiate groundsel and allowed a study of pre- and postzygotic breeding barriers between this new hybrid and its parent taxa.

Postzygotic breeding barrier

Postzygotic breeding barriers were found to exist between York radiate groundsel and both of its parents, *S. vulgaris* and *S. squalidus*. First generation hybrids between the tetraploid York radiate groundsel and diploid *S. squalidus* are triploid and highly sterile (see Chapter 3). Sterility in the hybrids presumably arises due to chromosome mispairing at meiosis and acts as a very effective postzygotic breeding barrier between the two taxa. In contrast, York radiate groundsel and *S. vulgaris* are both tetraploid and co-occur in York, and so there remains considerable potential for these two taxa to interbreed.

Experimental crosses between York radiate groundsel and *S. vulgaris* showed that F_2 progeny exhibited a significant reduction in male and female fertility compared to parental taxa. The average seed set in F_2 progeny was 58.8%; however, a great range of fertility was observed, such that some individuals exhibited 98% seed set while others produced no seed. Chromosome examination of a partially sterile F_2 individual revealed that, whilst the plant was tetraploid, on average two univalents were observed in pre-meiotic cells. It has already been established that York radiate groundsel possesses a substantial proportion of the *S. squalidus* genome (Chapter 2), and it is feasible that a few chromosomes may be composed mainly of genetic material derived from *S. squalidus*. This assertion is supported by meiotic chromosome observations of a triploid hybrid between York radiate groundsel and *S. squalidus*, in which, on average, two trivalents and a high proportion of bivalents were observed (Chapter 2). The formation of trivalents is believed to indicate chromosome homology (Ratter, 1973b). If York radiate groundsel possessed a portion of its chromosome complement derived from *S. squalidus*, then crosses with *S. vulgaris* would be expected to result in some chromosome mispairing. Recombination and segregation of chromosomes in the F_2 would produce individuals exhibiting a range of chromosome combinations, which should exhibit a range in fertility, such as that observed.

Prezygotic breeding barriers

Flowering time

Very few (if any) natural hybrids between York radiate groundsel and *S. vulgaris* were found at field sites in York. Initially, it was suspected that a difference in flowering time might be a cause of the observed lack of backcrossing. Although not complete, the difference in phenology was substantial. At Dalton Terrace, York, *S. vulgaris* plants flowered mainly from April to June whereas York radiate groundsel plants flowered from May to July, in 1993 and 1994. Flowering time is a complex trait affected by many characters, and in germination experiments it was found that *S. vulgaris* seeds germinated sooner than those of York radiate groundsel at high temperatures while field observations showed that *S. vulgaris* seedlings developed more rapidly in early spring than those of York radiate groundsel. Both observations would promote earlier flowering in *S. vulgaris* for seeds that were shed in late summer and which germinated before autumn to flower in late spring.

Modelling selection pressures on flowering time

If the difference in flowering time continued to increase between *S. vulgaris* and York radiate groundsel at Dalton Terrace then prezygotic reproductive isolation between these taxa, at this site, would be promoted by phenological separation. It was not possible to assess any long term change in flowering time at Dalton Terrace within the the period of PhD study; however, it is possible to establish whether selection might favour flowering time divergence from measurements of the relative fitness of plants which differ in flowering time. To this end, the field data from Dalton Terrace for 1994 were re-examined and the date that individuals first flowered was estimated, to the nearest week, from field notes, and expressed as the number of days from 1.1.94. The total number of capitula produced by each plant during the flowering season (estimated as the cumulative total of the number of capitula recorded on each sample date, Figure 4.3) was used as an estimate of individual fitness. On average, York radiate groundsel individuals first flowered after 72.1 days (from 1.1.94) while *S. vulgaris* first flowered after 43.9 days. A regression analysis of first flowering date against relative fitness (Figure 4.7) was not significant for *S. vulgaris* individuals (total number of capitula = 15.9 - 0.0638 . flowering date, $R^2 = 6.2\%$, $P = 0.108$), but there was a significant negative correlation for York radiate groundsel individuals (total number of capitula = 297 - 1.94 . flowering date, $R^2 = 59.2\%$, $P = 0.003$). Thus York radiate groundsel individuals that flowered earlier had a significantly higher fitness than late flowering plants. Consequently, should flowering time exhibit a high heritability in the material studied (as found in other plant species e.g. *Agrostis tenuis* and *A. odoratum*, McNeilly and Antonovics, 1968), selection would be expected to favour

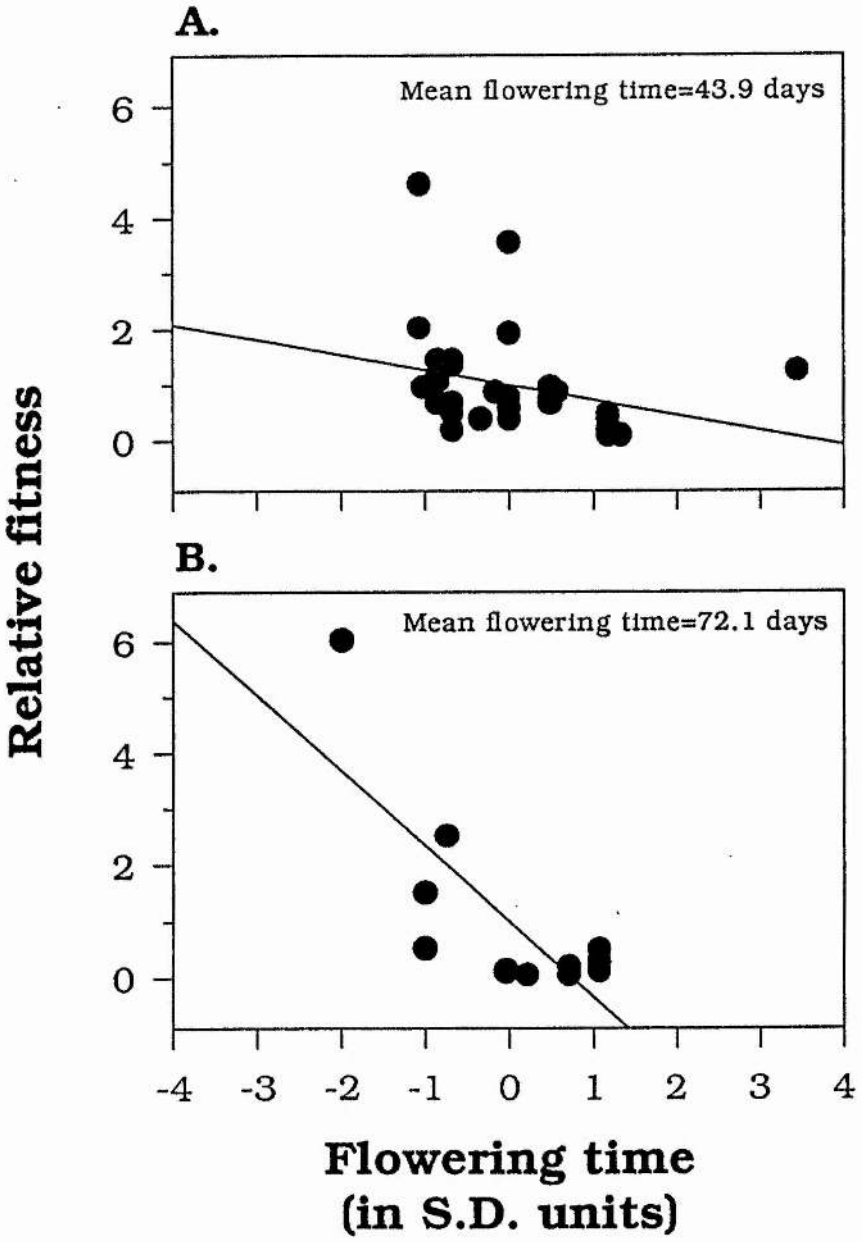


Figure 4.7. Plot of flowering time (expressed as standard deviation units around the mean number of days plants took to flower at Dalton Terrace from 1.1.94) against relative fitness (expressed as natural log of total number of capitula produced by each plant in a lifetime). Flowering time is expressed in SD units to allow easier comparison between plots. (a) for *S. vulgaris* var. *vulgaris*; (b) for York radiate groundsel.

earlier flowering York radiate groundsel plants in the population at Dalton Terrace rather than late flowering individuals. Thus convergence, rather than divergence of flowering time between the two taxa at Dalton Terrace, is the expected outcome of selection.

Although in theory, a difference in flowering time would be expected to secure reproductive isolation between two taxa it is probably not that important in preventing crosses between York radiate groundsel and *S. vulgaris* var. *vulgaris*. No intertaxon outcrossing was observed at any time of the year at York sites other than at Dalton Terrace, and even there, in 1993 the level of intertaxon crossing was very low (0 - 1.46% for York radiate groundsel plants, 0% for non-radiate groundsel plants). This low level of crossing was recorded despite the fact that York radiate groundsel and *S. vulgaris* plants often flowered profusely together when seed was collected. It seems, therefore, that a convergence of flowering time would not significantly increase intertaxon crossing and that differences in flowering time recorded in the field are probably not the main prezygotic breeding barrier between the two taxa.

Pollinator selection

The phenotype of *S. vulgaris* var. *vulgaris* suggests that it is predominantly autogamous (Gibbs, Milne and Vargas-Carillo, 1975) and previous estimates of outcrossing are typically below 1% (Marshall and Abbott, 1982, 1984a, 1984b; Irwin, 1990). In this study, intertaxon outcrossing in *S. vulgaris* var. *vulgaris* was zero at all sites in York. Estimates of female intertaxon outcrossing for York radiate groundsel was never greater than 1.46%, whereas field estimates of female intertaxon outcrossing in var. *hibernicus*, have been reported to be as high as 35% (Marshall and Abbott, 1984a). York radiate groundsel plants should not exhibit higher levels of autogamy than var. *hibernicus* as their capitula are much more 'showy', and they produce more pollen per floret (Chapter 2), which in turn should make them more attractive to pollinators and increase their level of outcrossing. The results of the garden experiment to investigate levels of intertaxon outcrossing showed that significantly more pollen was transferred from non-radiate to York radiate plants (18.3%) than from York radiate to non-radiate plants (1.4%). The results also showed that more pollen was transferred from *S. vulgaris* var. *hibernicus* to York radiate groundsel plants (10.2%) than from York radiate groundsel to var. *hibernicus* plants (2.8%). Unfortunately, the experiments did not separate out the component of outcrossing caused by the presence of pistillate ray florets. However, the ray florets make up, on average, 12.7% of the total number of florets present in a York radiate groundsel capitulum (eight out of 63), so at least some (5.6%) pollen transfer from *S.*

vulgaris to York radiate groundsel plants is probably due to their increased attractiveness. Also, York radiate groundsel and var. *hibernicus* plants both possess pistillate ray florets and so unequal pollen transfer between reciprocal crossing designs in experimental plots is probably also due to differential pollinator attractiveness of the two taxa.

Increased pollinator preference for York radiate groundsel over *S. vulgaris* plants would increase the ratio of pollinator preference calculated by Irwin and Abbott (0.62:0.28 radiate plants to non-radiate plants, 1988), and should lead to less transfers between non-radiate and radiate plants. To test this, another outcrossing experiment should be undertaken to include an estimate of inter- and intrataxon outcrossing for disc and ray florets, but the evidence from this garden experiment and observations from the field are consistent with the hypothesis that increased attractiveness of York radiate groundsel plants helps prevent backcrossing to *S. vulgaris*.

The overall level of intertaxon outcrossing in the garden experiment was much higher than that observed in the field and suggests that cross-pollination was boosted due to the close proximity of plants. An experimental plot design that places more distance between plants (e.g. one metre, as in the study by Abbott and Irwin, 1988, and Irwin, 1990), should reduce cross-pollinations and perhaps allow more accurate estimation of natural outcrossing frequency.

Capitulum characters that promote outcrossing

The existence of a self-incompatibility system acting in York radiate groundsel could promote outcrossing. The existence of such a self-incompatibility system has been postulated in *S. vulgaris* var. *hibernicus* (Marshall and Abbott, 1984b; Warren, Crawford and Oxford, 1988) but was effectively disproved by Abbott, Irwin and Forbes (1990). Similarly no self-incompatibility system was found to act in York radiate groundsel individuals as no difference was found in seed set between selfed and outcrossed capitula.

York radiate groundsel plants are visibly much more conspicuous to the eye than either variety of *S. vulgaris*, and morphometric analysis (Chapter 2) revealed that York radiate groundsel individuals possessed significantly more stigmatic papillae and pollen grains per floret than var. *hibernicus*. The mean phenotype of these characters was intermediate in York radiate groundsel between that of var. *vulgaris* and *S. squalidus*. The presence of stigmatic papillae can promote the capture of incoming pollen (Richards, 1986), and as pollen is a major food source for hoverflies (Gilbert, 1986),

Table 4.6. Means and significance of difference between York radiate groundsel (RR) and *S. vulgaris* var. *vulgaris* (NR), and radiate (R,R,) and non-radiate (N,R,) F_2 progeny for 15 capitulum characters.

Taxa	*S. vulgaris* v. *vulgaris*	York radiate groundsel		F_2 progeny		
			P (parental)	N,R,	R,R,	*P* (F_2)
N=	19	40	NR vs RR	27	20	N,R, vs R,R,
Character						
C2 Infloresence length[f] (cm)	1.221	1.78	***	1.65	1.68	ns
C3 Pedicel length[f] (cm)	0.51	0.97	***	0.90	0.89	ns
C4 Capitulum length (cm)	0.71	0.81	***	0.77	0.78	ns
C6 Number of phyllaries	18.9	15.3	***	19.0	17.9	ns
C8 Number of calyculous bracts	10.8	5.3	***	10.3	8.8	*
C9 Mean calyculous bract length (cm)	0.25	0.39	***	0.30	0.28	ns
C10 Number of ray florets	0.0	7.9	***	0	8.5	***
C11 Mean outer floret length[f] (cm)	0.18	0.42	***	0.19	0.42	***
C21 Mean seed length (cm)	0.22	0.29	***	0.25	0.26	*
C22 Total number of seeds per capitulum[f]	16.8	28.9	***	36.8	34.0	ns
C23 Number of pollen pores	3.0	4.00	***	3.31	3.39	ns
C26 Time to apical capitulum anthesis[f] (days)	36.7	36.3	ns	47.2	47.1	ns
C27 Number of pedicel bracts[f]	1.63	1.10	***	1.48	1.25	ns
C28 Total number of pollen grains[f]	270.4	632.0	***	439	524	*
C29 Number of stigmatic papillae[f]	5.6	21.1	***	12.5	18.3	*

[f] Log^e transformed.

the presence of copious pollen per floret should make York radiate groundsel plants very attractive to pollinators. To examine the possibility that capitulum characters promoting outcrossing in York radiate groundsel have been inherited from *S. squalidus* as a cosegregating block, the morphometric data collected from *S. vulgaris* var. *vulgaris* and York radiate groundsel and their F_2 progeny in Chapter 3 were re-analysed. The F_2 progeny were split into two categories, those which lacked ray florets and those which possessed full ray florets. F_2 plants that were heterozygous for the ray floret locus, and possessed short stubby rays, were omitted from analysis. Of the 39 characters originally measured, 15 described the capitulum (C2, C3, C4, C6, C8, C9, C10, C11, C21, C22, C23, C26, C27, C28 and C29). These 15 capitulum characters were re-analysed in turn by t-tests to examine first the difference between radiate non-radiate F_2 progeny and second differences between parental taxa (Table 4.6). Four characters (excluding those that described the number and length of ray florets) were significantly correlated ($P < 0.05$) with the ray floret locus in F_2 progeny (these were, C6, number of phyllaries; C21, mean seed length; C28, number of pollen grains per floret; and C29, number of stigmatic papillae), suggesting that they are controlled by genes tightly linked to the ray floret locus in York radiate groundsel plants. Thus, characters that could promote increased pollinator attraction of York radiate groundsel plants and which may cause ethological isolation from *S. vulgaris*, have been inherited from *S. squalidus* and have remained associated in York plants due to gene linkage.

Ecological factors affecting the establishment and maintenance of York radiate groundsel

York radiate groundsel co-occurred with *S. vulgaris* at most sites in York and was not found to exhibit any novel habitat preference. However, the two taxa differed significantly for several characters that may affect their respective ecologies. These factors included, germination behaviour, flowering time and seedling survival, which may, in turn, have important consequences for the establishment and maintenance of York radiate groundsel at site in York.

The germination behaviour of York radiate groundsel in the present study was found to be significantly different from *S. vulgaris* at most temperatures, and instead was much closer to that of *S. squalidus*. Although differences between the two taxa in speed of development were not significantly different when plants were raised under glass, at Dalton Terrace, *S. vulgaris* plants developed and flowered significantly earlier than those of York radiate groundsel, and the later flowering of York radiate

groundsel was more typical of *S. squalidus*. It would be of interest to establish in the future how these differences might act on the relative fitness of these taxa.

During winter and early spring at Dalton Terrace, *S. vulgaris* seedlings exhibited higher mortality than those of York radiate groundsel. This observation may be linked with pest damage suffered by both taxa as Warren (1987) has reported that *S. vulgaris* seedlings, raised under experimental conditions, were significantly more susceptible to predation by the slug *Deroceras reticulatum*, than were seedlings of radiate groundsel from York, and further that both groundsel types were grazed in preference to *S. squalidus*. Warren speculated that radiate plants from York may have inherited their slug resistance from *S. squalidus*. The fact that York radiate groundsel plants survived later than *S. vulgaris* into the flowering season (July and August), when conditions were relatively hot and dry, may reflect a greater tolerance to drought conditions. Warren (1987) reported that under conditions of water stress, radiate groundsel plants from York produced significantly more capitula, ovules and full seed in a life time than *S. vulgaris* individuals.

From the few studies conducted so far it would appear that York radiate groundsel may be sufficiently ecologically differentiated from its two parents such that it occupies a different niche from either of them. If this is so, then this may be of critical importance in affecting the establishment and maintenance of this new taxon at sites in the York area. It is clear, however, that much more detailed study needs to be conducted on the respective ecologies of York radiate groundsel and its two parent taxa before we can be sure how different the new taxon may be in this regard. Such detailed study was unfortunately beyond the scope of the present thesis.

Chapter 5.
Maintenance of York radiate groundsel in the wild: Inbreeding depression.

Introduction

Inbreeding depression, the reduction in fitness of progeny derived by inbreeding relative to outcrossing, and its converse, heterosis, the increased fitness of outcrossed progeny, have been well documented in natural and domesticated populations (Darwin, 1868; Wright, 1977; Charlesworth and Charlesworth, 1987). Inbreeding depression is considered the primary force opposing the two fold transmission advantage associated with self fertilization (Fisher, 1941), and if strong enough, will favour the evolution and maintenance of breeding systems promoting outcrossing (Lloyd, 1979; Charlesworth and Charlesworth, 1979; Lande and Schemske, 1985). There are two main genetic conditions that could cause inbreeding depression (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). First, overdominance, where the heterozygote at a particular locus exhibits greater fitness than either homozygote. Second, partial dominance, based on the accumulation of rare, deleterious, partially recessive alleles which are masked in the heterozygous condition but exposed to natural selection in the homozygous state. Under conditions of partial dominance, different inbred lines will become homozygous for different deleterious recessive genes and crossing between lines will promote outbreeding vigour (Maynard Smith, 1989).

Theoretical models

Many theoretical models have been advanced to predict the effects of selection on inbreeding depression in natural populations. Most models predict that, with some degree of dominance, inbreeding depression should decrease with increased inbreeding, as deleterious mutations of large effect become exposed to selection and are effectively purged from highly inbred populations (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1990). Alternatively, if recessive mutations are only mildly deleterious or have a low dominance coefficient, selection will be ineffective, relative to mutation, and substantial inbreeding depression may be maintained, even in highly inbred populations (Charlesworth, Charlesworth and Morgan, 1990; Charlesworth, Morgan and Charlesworth, 1990). When inbreeding depression is controlled by overdominance, it may increase temporarily following inbreeding (Charlesworth and Charlesworth, 1990; Charlesworth, Charlesworth and Morgan, 1990). However, unless the fitness of homozygotes at such loci is highly symmetrical, variation will eventually be lost, and the effects of overdominance are not expected to make a large contribution to the genetic

load of populations with a long history of inbreeding (Charlesworth and Charlesworth, 1987, 1990).

Inbreeding depression in plant populations

Recent work on natural populations (particularly in plants) has concentrated on testing the validity of some of the predictions of deterministic models (e.g. Holtsford and Ellstrand, 1990; Barrett and Charlesworth, 1991; Latta and Ritland, 1994; Husband and Schemske, 1995; Carr and Dudash, 1996). A recent review by Husband and Schemske (1996), has drawn together a comprehensive data set from studies of inbreeding depression in plant populations exhibiting a range of outcrossing rates, against which the main predictions of inbreeding depression models have been tested. A significant negative correlation between cumulative inbreeding depression and the primary selfing rate was found, and the average inbreeding depression in predominantly inbreeding species was significantly less than in predominantly outcrossing species (Husband and Schemske, 1996). These results support the hypothesis that recessive lethal and highly deleterious alleles are an important source of inbreeding depression in natural populations, which can be purged upon selfing (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Charlesworth, Morgan and Charlesworth, 1990; Barrett and Charlesworth, 1991; Husband and Schemske, 1995; Husband and Schemske, 1996). Secondly, Husband and Schemske (1996) found a significant relationship between the stage at which inbreeding depression was expressed in the life cycle and breeding system. Most self-fertilizing species expressed inbreeding depression late in the life cycle (growth and reproduction) while, outcrossing species expressed it either early (seed production) or late. These results support the hypothesis that most early-acting inbreeding depression is due to recessive lethal mutations of large effect that can be purged through inbreeding (Lande and Schemske, 1985), whereas much of the late-acting inbreeding depression is due to weakly deleterious mutations that are difficult to purge, even under extreme inbreeding.

Inbreeding depression in polyploids and hybrids

The success and vigour of autopolyploids has been partially attributed to heterozygosity and heteroallelism that confer biochemical and physiological advantages (Stebbins, 1980; Soltis and Soltis, 1993). However, inbreeding in autopolyploids would increase homozygosity and, under these polysomic conditions, reduce fitness (Barrett, 1989). In contrast, heterozygosity in allopolyploids results from gene multiplication (fixed heterozygosity), and the high biochemical diversity will not be broken down by inbreeding (Barrett, 1989). Theoretical models indicate that the magnitude of inbreeding depression can be reduced by population bottlenecks and a similar effect can also arise

from the restricted origin of allopolyploid taxa, due to a 'hybridity bottleneck' (Lande and Schemske, 1985; Hedrick, 1987). As a result of the decreased genetic load in newly established allopolyploids, genes that increase the rate of selfing would be expected to increase in frequency (Hedrick, 1987). Indeed, assured reproduction through selfing is common in allopolyploids (Barrett, 1989), and the combination of fixed heterozygosity and autogamy has been proposed as an important feature responsible for the success of many allopolyploid weeds (Barrett and Shore, 1989).

Mating systems in *Senecio*

Senecio vulgaris var. *vulgaris* is a predominant inbreeder (Marshall and Abbott, 1982) with many features associated with a well established selfer (Gibbs, Milne and Vargas-Carillo, 1975). In contrast, *S. squalidus* has a very effective self-incompatibility system (Gibbs, Milne and Vargas-Carillo, 1975), probably of the homomorphic sporophytic type, and normally exhibits random outcrossing in British populations (Abbott and Forbes, 1993). The introgression of the ray floret gene into *S. vulgaris* var. *hibernicus* from *S. squalidus* has significantly increased the rate of outcrossing in *S. vulgaris* (Marshall and Abbott, 1982, 1984a, 1984b). Increased outcrossing exhibited by var. *hibernicus* is mainly due to functional protogyny in the ray florets (Marshall and Abbott, 1984a, 1984b) and its increased attractiveness to pollinators compared to var. *vulgaris* (Abbott and Irwin, 1988; Irwin, 1990). Marshall and Abbott (1984a, 1984b, 1986) have argued that the increased outcrossing exhibited by var. *hibernicus* should lead to selection against the radiate allele, in natural populations, due to the cost of outcrossing (Fisher, 1941). Oxford and Andrews (1977) showed that in some mixed populations, radiate plants produce significantly more seeds per plant than non-radiate plants, and such increased reproductive success would be enough to outweigh the 'auto-selective' advantage of the non-radiate allele (Marshall and Abbott, 1986). However, in other populations no reproductive advantage was found for var. *hibernicus* (Marshall and Abbott, 1986) and in these locations the radiate allele must be maintained by other means. Inbreeding depression is a factor that could promote the maintenance of the radiate allele in groundsel populations. However, due to the highly inbred nature of groundsel, inbreeding depression is unlikely, and what is more, its effects have never been observed even in highly inbred glasshouse lines of var. *hibernicus* (Abbott, 1985; D. Forbes, personal communication). Further work on the ecology of groundsel has shown that the maintenance of var. *hibernicus* may be associated with complex ecological factors rather than factors only associated with the maintenance of the radiate allele (Richards, 1975; Kadereit and Briggs, 1985; Abbott, 1986; Warren, 1987; Abbott, Horrill and Noble, 1988; Abbott and Horrill, 1991; Briggs and Block, 1992; Theaker and Briggs, 1992).

Objectives

Results in Chapter 4 suggest that York radiate groundsel exhibits a higher level of outcrossing than *S. vulgaris*, and that sites where populations occur in York may be subject to occasional disturbance that may drastically reduce numbers. It is possible that inbreeding depression may act to reduce fitness in York radiate groundsel populations, particularly in those that undergo severe reduction in numbers where individuals would be forced to reproduce predominantly by autogamy. Indeed, D. Forbes (personal communication) has noted that after several generations of selfing under glass, some York radiate groundsel plants failed to set seed. To examine the effects of inbreeding depression in groundsel, several inbred generations of York radiate groundsel, *S. vulgaris* var. *vulgaris* and var. *hibernicus* were produced and their male and female fertility compared to individuals raised from outcrossed seed.

Methods

Open pollinated seed was collected from five randomly sampled individuals of each of *S. vulgaris* var. *vulgaris* and York radiate groundsel from Dalton Terrace, York, and var. *hibernicus* from Edinburgh. Seed from each field individual were used to establish five glasshouse lines of each of the three taxa, and the overall experimental design was similar to that used by Barrett and Charlesworth (1991).

On a single individual chosen from each line, several capitula were randomly outcrossed to other lines within the same taxon (see Chapter 3 for emasculation technique) and several immature capitula were covered with bags made from tissue paper to establish the first outcrossed (O1) and first selfed (S1) generations, respectively. Seed collected from capitula treated in this way, and those from subsequent generations, were stored at 4^{o} C for a maximum of 2 years until used. Seed germination and propagation were as described in Chapter 2. A small amount of S1 seed from each line was sown out and a single individual from each line was chosen at random and selfed to produce an S2 generation. The process was repeated with S2 seed to produce an S3 generation. A small amount of S3 seed from each line was sown out and a single individual was selected from each line and randomly outcrossed between lines and selfed, as previously described, to generate O4 and S4 lines respectively. Up to three seeds of each line/taxa/generation (P1, S1, O1, S2, S3, S4, O4) were sown out and a total of 259 individuals were raised together in a fully randomized design.

On each individual, pollen fertility was assessed on grains taken from disc florets of the second-most apical capitulum, and calculated as the proportion of pollen grains, out of 100, that took up aceto-carmine stain. The proportion of seed set was assessed by covering two unopened capitula with tissue paper bags, and leaving achenes to mature. Once ripe, the proportion of seed set and total number of florets per capitulum was averaged over capitula for each individual. Finally, each plant was left to mature and die off, at which point the total number of capitula produced was counted.

In addition, the total number of seeds produced in a life time was estimated as the product of proportion seed set, number of florets per capitulum and total number of capitula produced in a life time. The normality and heteroscedasticity of data was assessed and those not conforming were transformed before one-way ANOVA and Tukey-Kramer multiple comparison. For several lines of each taxon, not enough seed was produced for the outcrossed generations (O1 and O4), and so data from all lines was pooled for each generation before analysis.

Results

Although not measured quantitatively, no difference in seed germination or seedling survival was observed between generations or taxa. Means for each line and taxon per generation for pollen fertility, proportion of seed set, number of florets per capitulum and total number of capitula produced in a lifetime are presented in Table 1, along with the results of Tukey-Kramer multiple comparison tests. The estimated total lifetime production of seed for each taxon per generation is shown in Figure 1.

For all measures of fitness and fertility, no taxon exhibited a significant difference between O1 and S1 or O4 and S4 generations. There were, however, some differences between other generations within taxa. For *S. vulgaris* var. *vulgaris* and var. *hibernicus*, no significant reduction in the proportion of seed set, total number of seed per capitulum, total number of capitula produced or total number of seeds produced per lifetime was observed for any inbred or outbred generation compared to open pollinated parental plants, although S2 and S3 lines of var. *vulgaris* produced significantly more capitula in a life time than parental plants. For York radiate groundsel the S4 and O4 generations exhibited significantly fewer seeds per capitulum and total number of capitula than parental plants; however, there was no significant difference for other fitness measures. For pollen fertility, the O4 generation was significantly lower than parental plants for all taxa, and for var. *hibernicus* and York radiate groundsel this was also the case for the S4 generation. The O1, S1 and S2 generations of *S. vulgaris* var. *vulgaris* also exhibited significantly lower pollen fertility than parental plants, but were not significantly different from each other or the O4 generation.

Table 5.1. Line and taxon means for pollen fertility, percentage seed set, total number of florets per capitulum and total number of capitula produced in a lifetime measured on inbred and outcrossed generations of *S. vulgaris* var. *vulgaris*, var. *hibernicus* and York radiate groundsel. Most line means are derived from three individuals, for other lines the number of plants examined is indicated in parentheses below the line mean. Standard deviations of the taxon means are shown in parentheses below means. Results of Tukey-Kramer multiple comparison are presented with taxon means where generations sharing the same superscript are not significantly different (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns-not significant).

Pollen fertility (%)[f]

	S. vulgaris var. *vulgaris*						*S. vulgaris* var. *hibernicus*						York radiate groundsel					
Line	1	2	3	4	5	mean	1	2	3	4	5	mean	1	2	3	4	5	mean
Generation																		
P1	94.3	96.1	97.9	95.8	97.1	**96.3**^{a} (2.8)	94.5	99.6	97.7	94.6	95.2	**96.3**^{a} (4.0)	93.9	94.4	91.9	98.9	89.1	**93.7**^{a} (5.8)
O1	81.0 (2)	69.1 (1)	-	73.5 (2)	74.3 (1)	**75.4**^{d} (4.8)	-	-	-	100.0 (2)	94.5 (1)	**98.2**^{a} (3.1)	82.2	89.5	93.9	93.4 (1)	93.8	**90.1**^{ab} (6.2)
S1	78.6	81.1	94.7 (1)	79.9 (2)	77.8	**80.6**^{dc} (9.9)	94.9	90.3	92.5	94.8 (2)	91.1	**92.6**^{ab} (5.3)	82.2	94.4	92.2	93.7	41.9	**80.9**^{ab} (22.7)
S2	91.8	87.6	80.8	83.8 (2)	62.9	**81.2**^{bcd} (16.6)	97.9	84.5	92.1	97.6	95.0	**93.4**^{ab} (8.8)	92.8	71.5	92.7	88.1	94.6	**87.9**^{ab} (15.2)
S3	98.4	98.5	82.7	89.0	80.0	**89.7**^{abc} (10.9)	91.6	81.1	74.6	98.4	78.0 (1)	**85.8**^{ab} (16.1)	79.9	64.3	82.6	84.8	66.5	**75.6**^{bc} (23.2)
S4	100 (2)	96.8	-	87.4 (2)	90.1	**93.8**^{ab} (5.6)	73.1	91.2	54.6 (2)	-	85.7 (2)	**77.3**^{b} (18.4)	72.9	72.4	56.9	68.2	48.2	**63.7**^{c} (24.2)
O4	78.2 (2)	86.9	54.0 (1)	-	-	**78.5**^{bcd} (16.4)	84.1	88.9	81.6 (2)	86.5 (1)	59.2	**78.9**^{b} (18.8)	84.1	75.0 (1)	77.3	65.2 (2)	82.9 (2)	**77.8**^{bc} (10.4)
P						***						***						***

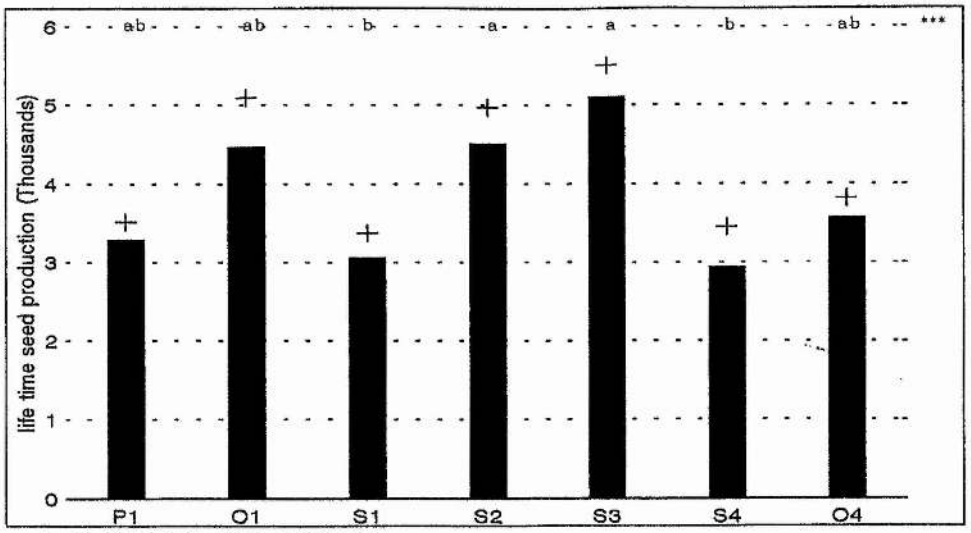
Percentage seed set (%)[f]

P1	96.9	92.9	86.1	91.7	96.2	**92.8**^{ab} (5.7)	92.7	90.3	94.9	71.7	89.4	**87.8** (10.4)	66.2	68.5	46.3	46.2	52.7	**56.0** (20.9)
O1	95.5 (2)	96.5 (1)	-	90.8 (2)	95.6 (1)	**94.1**^{ab} (4.2)	-	-	-	86.9 (2)	91.3 (1)	**88.4** (8.4)	38.2	61.2	78.4 (1)	75.9	80.5	**65.5** (23.8)
S1	82.9	78.2	92.8 (1)	96.9 (2)	95.4	**88.1**^{ab} (14.4)	93.9	97.1	93.3	79.3 (2)	94.8	**92.6** (6.5)	82.6	81.7	34.4	65.5	25.6	**57.9** (29.4)
S2	84.9	92.7	96.7	97.8 (2)	80.7	**90.0**^{ab} (10.1)	92.7	93.3	96.2	70.6	93.4	**89.3** (10.3)	77.9	66.7	56.6	76.7	75.9	**70.8** (12.4)
S3	94.6	94.6	96.2	91.0	93.2	**93.9**^{a} (3.1)	82.5 (2)	83.6	76.5	70.5	88.7 (2)	**79.6** (16.9)	74.1	80.9	81.3	24.4	47.7	**61.7** (24.6)
S4	83.3	94.3	-	61.4 (2)	84.8	**82.7**^{b} (14.0)	96.4	93.3 (2)	91.9 (2)	-	76.2 (2)	**90.2** (13.2)	68.6 (2)	48.7	78.8	46.3	68.5	**61.7** (21.9)
O4	87.8 (2)	90.2	21.0 (1)	-	-	**77.8**^{b} (28.1)	80.5 (2)	71.0	94.3	86.3 (1)	82.4	**82.5** (20.3)	76.8 (2)	-	64.9	74.2 (2)	71.4 (2)	**71.1** (16.1)
P						*						ns						ns

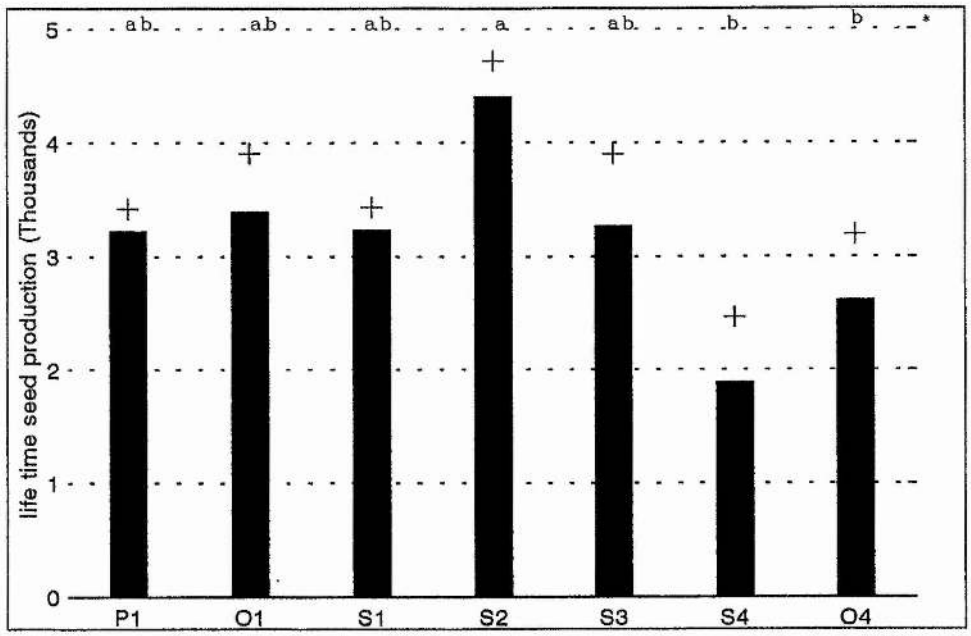
Table 5.1. Continued.

Line	*S. vulgaris* var. *vulgaris*						*S. vulgaris* var. *hibernicus*						York radiate groundsel					
Generation	1	2	3	4	5	mean	1	2	3	4	5	mean	1	2	3	4	5	mean
Total number of florets per capitulum[g]																		
P1	59.5	53.8	57.5	63.2	61.0	**59.0** (5.6)	52.7	48.2	52.0	53.0	48.8	**50.9** (3.9)	54.8	60.2	59.8	48.7	61.2	**56.9**^{a} (6.5)
O1	61.5 (2)	57.5 (1)	-	61.5 (2)	69.0 (1)	**62.1** (3.9)	-	-	-	52.5 (2)	52.0 (1)	**52.3** (7.5)	51.7	57.2	59.2	68.5 (1)	68.3	**59.8**^{a} (8.2)
S1	60.7	55.3	63.0 (1)	64.7 (2)	57.8	**59.5** (6.4)	53.7	46.7	46.2	53.2 (2)	53.3	**50.4** (4.9)	59.8	62.0	59.2	60.5	60.8	**60.5**^{a} (2.3)
S2	55.2	47.7	45.2	46.0 (2)	65.5	**52.3** (10.4)	52.0	53.0	49.8	50.5	48.2	**50.7** (3.6)	57.2	54.3	58.5	53.2	51.0	**54.8**^{a} (5.9)
S3	50.3	52.0	61.3	57.3	57.0	**55.6** (6.3)	48.2 (2)	50.2	52.2	47.0	50.7 (2)	**49.7** (4.4)	56.7	60.5	57.0	49.7	53.5	**55.5**^{a} (6.6)
S4	48.0	49.5	-	61.7 (2)	62.7	**54.9** (11.2)	41.7	44.0 (2)	45.7 (2)	-	51.2 (2)	**45.2** (8.1)	49.2 (2)	38.2	42.3	48.5	51.8	**45.8**^{b} (9.8)
O4	45.0	60.5 (1)	47.5	-	-	**53.2** (9.8)	57.0 (2)	55.8	51.7	58.5 (1)	45.5	**52.6** (6.3)	37.2 (2)	-	40.7	46.0 (2)	41.2 (2)	**41.2**^{b} (6.6)
P						ns						ns						***
Total number of capitula produced in a lifetime[g]																		
P1	57.3	62.0	51.0	73.7	53.7	**59.5**^{b} (13.3)	73.7	67.0	72.0	74.7	74.7	**72.4**^{ab} (14.1)	80.0	64.3	44.0	43.7	89.3	**64.3**^{ab} (23.4)
O1	63.0 (2)	54.0 (1)	-	92.0 (2)	92.0 (1)	**76.0**^{ab} (23.8)	-	-	-	66.5 (2)	85.0 (1)	**72.7**^{ab} (10.7)	55.7	57.7	57.0	37.0 (1)	49.7	**53.6**^{bc} (10.1)
S1	68.3	46.3	71.0 (1)	39.5 (2)	63.7	**57.1**^{b} (17.2)	77.3	71.3	76.3	63.5 (2)	59.7	**70.1**^{ab} (17.3)	48.7	45.7	61.0	45.3	63.7	**52.9**^{bc} (15.4)
S2	96.3	91.5 (2)	103.3	100.5 (2)	92.7	**97.0**^{a} (29.4)	97.7	110.0	105.3	80.7	87.0	**96.1**^{a} (18.9)	51.7	73.7	77.7	64.7	54.0	**64.3**^{ab} (17.1)
S3	93.3	116.3	60.7	107.0	113.7	**98.2**^{a} (28.3)	98.0 (2)	52.7	82.0	131.5 (2)	52.5 (2)	**80.7**^{ab} (39.8)	85.0	86.3	91.3	89.7	72.3	**84.9**^{a} (12.6)
S4	45.0 (2)	53.3	-	83.5 (2)	73.7	**63.8**^{b} (19.3)	55.7	43.5 (2)	31.0 (1)	-	-	**47.5**^{bc} (43.3)	45.5 (2)	40.3	37.3	69.7	31.7	**44.9**^{c} (22.6)
O4	69.0 (2)	74.0	-	-	-	**72.0**^{ab} (16.1)	43.5 (2)	101.3	55.3	10.0 (1)	52.7	**60.4**^{b} (39.4)	34.5 (2)	78.0 (1)	43.3	32.5 (2)	49.5 (2)	**44.1**^{c} (24.1)
P						***						***						***

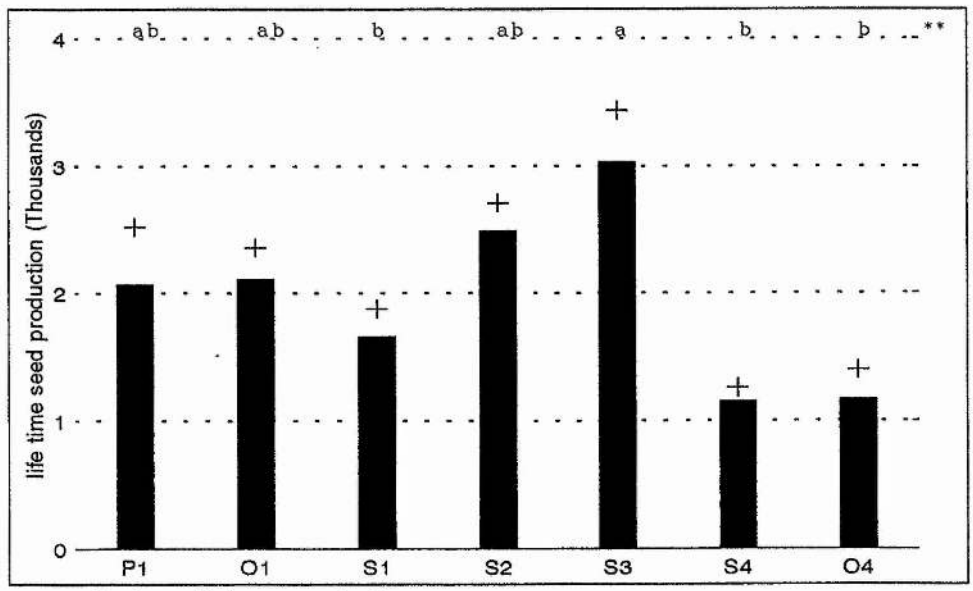
[1] arcsine transformed, [g] loge transformed



a. S. vulgaris var. vulgaris



b. S. vulgaris var. hibernicus



c. York radiate groundsel

Figure 5.1. Plot of mean life time seed production in inbred and outcrossed generations for particular taxa. Crosses indicate extent of one SD. Results of Tukey-Kramer multiple comparison are presented above bars (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

Discussion

No significant reduction in any measures of fertility or fitness was observed between O1 and S1 or between O4 and S4 generations for York radiate groundsel, *S. vulgaris* var. *vulgaris* or var. *hibernicus*. Differences in fitness between inbred and outbred lines are normally taken to indicate inbreeding depression. Therefore, no significant effect of inbreeding depression was found to be acting in any of these taxa late during their life cycle (capitula production, seed set and pollen fertility), when weakly deleterious mutations that are difficult to purge, even under extreme inbreeding, would be expected to act (Husband and Schemske, 1996).

The predominantly inbreeder *S. vulgaris* var. *vulgaris* (Marshall and Abbott, 1982, 1984a) is thought to be of allopolyploid origin (Weir and Ingram, 1980; Ashton and Abbott, 1992b) and exhibits fixed heterozygosity for some isozyme systems (Ashton and Abbott, 1992a, b). A combination of a highly autogamous breeding system and a probable bottleneck associated with an allopolyploid origin (Hedrick, 1987), should be sufficient to have purged most deleterious recessive mutations from the var. *vulgaris* genome and thus it is not surprising that no inbreeding depression was observed.

S. vulgaris var. *hibernicus* exhibits increased outcrossing due to the introgression of the ray floret gene from *S. squalidus* (Marshall and Abbott, 1982a, 1984a,b; Abbott and Irwin, 1988). However, the frequency of outcrossing is still usually below 15% (Marshall and Abbott, 1982, 1984a). *S. vulgaris* var. *hibernicus* also exhibits fixed heterozygosity for some isozyme loci (Ashton and Abbott, 1992a, b), and has low levels of isozyme diversity, which is associated with its restricted origin (Abbott, Irwin and Ashton 1992). Thus, it is not surprising that this taxon also exhibits no significant effects of inbreeding depression.

York radiate groundsel is also a hybrid product which has certainly undergone a 'hybridity bottleneck'. No isozyme diversity was observed between any York populations of this taxon and several loci potentially exhibited fixed heterozygosity (*Aat-1* and *Idh-1*, Chapter 2 and 3). Although the influence of the ray floret locus, and other genes associated with pollinator attraction (Chapter 4), should increase the level of outcrossing above that normally exhibited by var. *vulgaris*, York radiate groundsel probably remains predominantly inbreeding. As no effects of inbreeding depression were exhibited by York radiate groundsel, it is likely that the effects of small population size and predominant autogamy should not present a problem for its continued survival under such circumstances.

The level of inbreeding depression acting in *S. squalidus* was not measured. Although one would expect inbreeding depression in the diploid outcrosser, there is good evidence that its origin in Britain involved a severe bottleneck. For about 100 years a few individuals of *S. squalidus* were cultivated at the Oxford Botanic Gardens before it started to spread throughout the British Isles (Druce, 1927; Kent, 1956). The sporophytic self-incompatibility system survived and so at least 4 S-alleles (in the absence of dominance) must have been introduced into the British population (Abbott and Forbes, 1993), which would require only two individuals. Under some conditions, the effects of biparental inbreeding can be as severe as autogamy (in *Gaillardia pulchella*, Heywood, 1993; in *Raphanus sativus*, Nason and Ellstrand, 1995), and may have allowed a certain amount of purging of deleterious recessives from British *S. squalidus* material. It would be of interest to examine the level of inbreeding depression exhibited by *S. squalidus* in the light of the significant bottleneck involved in its origin.

In the absence of inbreeding depression, the auto-selective advantage of the non-radiate allele (Marshall and Abbott, 1986) may favour the spread of a non-radiate variant of York radiate groundsel. However, a number of ecological factors are associated with York radiate groundsel that may help to maintain the radiate allele, as is the case with var. *hibernicus* (Marshall and Abbott, 1986). A small population of non-radiate groundsel exists near the Spotted Cow pub in York, that bear a close morphological resemblance to York radiate groundsel and possesses the distinctive β*Est-1a* allele. If further investigation into the selection pressures that maintain the radiate allele in York radiate groundsel were to be undertaken, it would be worth including some individuals from this population in the analysis.

Chapter 6.
General Discussion

Hybridization is recognized as an important process in the evolution of plant taxa (Anderson, 1949; Anderson and Stebbins, 1954; Stebbins, 1959; Stace, 1975; Levin, 1979; Grant, 1981). The study of hybrid taxa allows insights into the processes that generate such species, although it is often difficult to discern the exact history of events (Harrison, 1991) and at best we can only indicate which are plausible or at most likely (Lewontin and Birch, 1966). Examples of recently evolved hybrid taxa offer the best opportunity to discover the processes involved in hybridization, as the distinctive 'footprints' that may be left behind by the formative processes are not as likely to be obscured by subsequent changes (Avise, 1994). Such an opportunity was presented by the discovery of York radiate groundsel in 1979 soon after its presumed origin. By asking precise questions and using suitable techniques, it seemed possible to unravel the processes that lead to its formation and affect its maintenance.

The results of morphometric, isozyme, mitotic chromosomal, rDNA and RAPD analyses, presented in Chapter 2, strongly suggest that York radiate groundsel is a hybrid product between *S. vulgaris* var. *vulgaris* and *S. squalidus*, supporting the original assertion of Irwin and Abbott (1992). Information from meiotic chromosome pairing behaviour and artificial crossing studies (Chapter 3) are consistent with other lines of evidence in suggesting that York radiate groundsel exhibits a genomic constitution that is more similar to *S. squalidus* than is *S. vulgaris*. The results also clearly indicate that York radiate groundsel is distinct from the stabilized introgressant *S. vulgaris* var. *hibernicus*, as it exhibits significantly more *S. squalidus* genetic markers than are present in the latter. In addition, morphological, isozyme and rDNA evidence suggest that York radiate groundsel is not a first generation hybrid between its parental taxa, but has probably undergone limited backcrossing to *S. vulgaris* to produce the pattern of marker variation observed. Morphological and isozyme analysis (Chapters 2 and 3) could not differentiate the two main populations of York radiate groundsel examined and, in conjunction with cpDNA evidence, suggest that extant populations of York radiate groundsel are probably derived from a single origin.

Five pathways that lead to the formation of tetraploid radiate groundsel were originally postulated by Crisp (1972), and although it may not be possible to absolutely distinguish between the different routes, evidence to suggest the most likely can be obtained. The possible routes are:

1. Hybridization between *S. cambrensis* and *S. squalidus.*
2. A reduction in chromosome number from the hexaploid *S. cambrensis* to a tetraploid radiate groundsel plant.
3. Hybridization between tetraploid *S. squalidus* and *S. vulgaris.*
4. Fusion of an unreduced gamete of *S. squalidus* with a reduced gamete of *S. vulgaris.*
5. Hybridization between *S. vulgaris* and *S. squalidus,* followed by backcrossing of the triploid F_1 hybrid to *S. vulgaris,* or segregation in the F_2, with resumption of tetraploidy.

Routes 1 and 2 are not considered likely for the origin of York radiate groundsel as *S. cambrensis* has never been reported in the York area. However, hybrid progeny, similar in morphology to York radiate groundsel and *S. vulgaris* var. *hibernicus,* were generated artificially by routes 3, 4 and 5 (Chapter 3). It was notable that hybrid progeny generated after backcrossing to *S. vulgaris,* were more likely to be similar to one of these two taxa than were F_2 or F_3 progeny. Further backcrossing of such hybrid progeny to *S. vulgaris* would probably be necessary to produce plants that were identical to *S. vulgaris* var. *hibernicus* in morphology and genotype, but is probably not required to produce the more intermediate phenotype of York radiate groundsel.

Further scrutiny revealed that route 3 could be excluded as a likely pathway due to previous failures to find tetraploid *S. squalidus* growing in natural populations of the species. Route 4 depends on the ability of diploid *S. squalidus* to produce unreduced gametes that fuse with normally reduced gametes of *S. vulgaris.* This is shown to be possible, but tetraploid F_1 hybrids are produced much less frequently than triploid hybrids following crosses between diploid *S. squalidus* and tetraploid *S. vulgaris.* However, the tetraploid hybrids exhibit much higher fertility than the triploids. Route 5, involving the production of balanced diploid gametes by the F_1 hybrid has been the most favoured pathway of origin of radiate groundsel in the past (see Ingram, Weir and Abbott, 1980). However, the production of fertile progeny via this route requires two steps. Firstly, the production of *S.* x *baxteri,* which occurs regularly, but at low frequency (Marshall and Abbott, 1980). Secondly, the production of later generation, fertile progeny either via backcrossing or by segregation is shown to be possible (Chapter 3; Crisp, 1972; Ingram, 1978; Ingram, Weir and Abbott, 1980), but is likely to be a rare event in the wild. Consequently, it is concluded that route 4 may be more likely to have been the route of origin of York radiate groundsel relative to route 5.

Recent reviews have shown that the production of unreduced gametes by diploid taxa has played an important role in the origin of many auto- and allopolyploid species (Harlan and deWet, 1975; Thompson and Lumaret, 1992; Bretagnolle and Thompson, 1995). The production of unreduced gametes is likely to have been important in the origin of *S. cambrensis* (Weir and Ingram, 1980), and may have also been instrumental in the origin of *S. vulgaris* var. *hibernicus* and York radiate groundsel.

A survey of herbarium material revealed that semi-fertile hybrids between *S. vulgaris* and *S. squalidus* have arrisen previously in the British Isles, some being very similar in morphology to York radiate groundsel. It is important to consider why the York population of hybrid radiate groundsel has become established and maintained itself successfully while other individuals/populations did not. After visiting other locations where hybrid plants have been recorded, the main reason for their loss was most probably linked with habitat loss, although there is evidence that some hybrid populations may have been 'genetically eroded', following backcrossing to *S. vulgaris*. A number of sites where York radiate groundsel was established around York in the 1980's have been lost to development schemes, but several other sites have remained unchanged and still support populations. At these sites, York radiate groundsel does not seem to have backcrossed to its parental taxa, and has maintained its distinctive morphological phenotype since at least 1979 (Chapter 2; Warren, 1987; Irwin and Abbott, 1992), and its distinctive isozyme phenotype since 1991 (Chapter 2; Irwin and Abbott, 1992), suggesting that it is reproductively isolated from its parents.

Postzygotic breeding barriers were found to exist between York radiate groundsel and both of its parents, *S. vulgaris* and *S. squalidus*. First generation hybrids between the tetraploid York radiate groundsel and diploid *S. squalidus* are triploid and highly sterile (Chapter 3). Sterility in the hybrids presumably arises due to chromosome mispairing at meiosis and acts as a very effective postzygotic breeding barrier between the two taxa. York radiate groundsel and *S. vulgaris* var. *vulgaris* are both tetraploid, but crosses between them yielded F_2 progeny that exhibited a significant reduction in male and female fertility compared to parental generations (Chapter 4). It is most likely that York radiate groundsel possesses some chromosomes that contain a significant portion of genetic material derived from *S. squalidus*, that causes infertility when crossed with *S. vulgaris*, due to some chromosome mispairing. Very few tetraploid hybrids have been reported in the literature that have been produced by crossing diploid and tetraploid taxa, and the observed breeding barrier between York radiate groundsel and *S. vulgaris* is, therefore, of considerable interest. Ratter (1972) described the generation of a fertile tetraploid hybrid following crosses between

Spergularia nicaeensis (4x = 2n = 36) and *S. purpurea* (2x = 2n = 18), via a triploid F_1. The fertile tetraploid hybrid progeny were, however, also interfertile with the tetraploid parent. Postzygotic sterility in diploid hybrids has been attributed to the recombination in the hybrid of chromosome segments that distinguish the parental taxa (Grant, 1966; Stebbins and Daly, 1961; Gallez and Gottlieb, 1982; Arnold, Hamrick and Bennett, 1990; Rieseberg , 1991; Rieseberg and Wendel, 1992). The postzygotic chromosomal breeding barrier between York radiate groundsel and *S. vulgaris* does have certain similarities to this recombination breeding barrier and may also be of importance in the origin of polyploid taxa following heteroploid crosses.

Besides the strong postzygotic breeding barrier between York radiate groundsel and *S. squalidus*, these taxa do not occur together at many sites in York and so further isolation barriers between the taxa were not considered. York radiate groundsel and *S. vulgaris* co-occur at most sites in York, but hybrid individuals between the taxa have only been recorded at one site in York (Dalton Terrace) and even then are very rare. In addition, progeny testing of 3702 field pollinated seed, collected from 401 individuals over 2 years from different sites around York, only revealed three intertaxon crossing events. A difference in flowering time between *S. vulgaris* and York radiate groundsel is observed in the field, with York radiate groundsel plants flowering later in the season. However, further examination of selection pressure on flowering time change, revealed that a convergence, not divergence, of flowering time is the expected outcome of selection. In conjunction with the fact that very few, if any, hybrid progeny are produced even during periods when the two taxa are flowering profusely together, it seems probable that flowering time is not the main prezygotic isolating mechanism between York radiate groundsel and *S. vulgaris*.

In a garden experiment, significantly more intertaxon hybrids were recorded when York radiate groundsel plants were surrounded by *S. vulgaris* var. *vulgaris* or var. *hibernicus* plants, than in the reciprocal arrangement. These results indicate that York radiate groundsel may be more attractive to pollinators than either variety of *S. vulgaris*. If this observation is confirmed, it is expected that the ratio of pollinator preference of radiate to non-radiate plants calculated by Irwin and Abbott (0.62:0.28, 1988) should increase, leading to less pollen transfers between non-radiate and radiate plants and effective ethological reproductive isolation. It was further found that characters that could promote increased pollinator attraction and outcrossing in York radiate groundsel compared to either variety of *S. vulgaris* (more pollen per disc floret and presence of stigmatic papillae), have probably been inherited from *S. squalidus*

and have remained associated with the ray floret locus in York plants due to gene linkage.

York radiate groundsel and *S. vulgaris* differ significantly for several ecological characters including, germination behaviour, flowering time and seedling survival. Although results are preliminary, they suggest that York radiate groundsel may be sufficiently ecologically differentiated from its two parents such that it occupies a different niche from either of them. If this is so, then this may be of critical importance in affecting the establishment and maintenance of this new taxon at sites in the York area.

No significant effect of inbreeding depression was exhibited by *S. vulgaris* var. *vulgaris*, var. *hibernicus* or York radiate groundsel during the reproductive stage of the life cycle, when weakly deleterious mutations are expected to act in inbreeding populations (Husband and Schemske, 1996). Due to its recent hybrid origin, York radiate groundsel has certainly undergone a 'hybridity bottleneck' (Hedrick, 1987; Barrett, 1989) and so the lack of inbreeding depression is not surprising. Several sites in York experience considerable disturbance due to weeding, flooding and periodic redevelopment, which may lead to a reduction in population size. Propagation by autogamy in such situations, should therefore not lead to the increased deleterious effects of inbreeding depression. It was noted that weeding pressure is increasing at most sites around York and although weed maintenance keeps sites clear of more competitive weeds, allowing repeated groundsel establishment in successive years, this pressure may eventually threaten populations of York radiate groundsel.

The taxonomy of hybrids has always been a difficult subject (Stace, 1980) and presents particular problems for those wishing to define species. According to the biological species concept, York radiate groundsel should be recognized as a new species due to its reproductive isolation from *S. vulgaris* and *S. squalidus*. York radiate groundsel also possess a distinct morphology that makes it easily distinguishable in the wild from varieties of *S. vulgaris*. However, both York radiate groundsel and *S. vulgaris* are tetraploid and although the reticulate evolutionary lineage of York radiate groundsel distinguishes it from var. *vulgaris*, var. *hibernicus* is also a product of hybridization with *S. squalidus* and York radiate groundsel may just be considered an extreme variant of *hibernicus* (Warren, 1987; Oxford, Crawford and Pernyes, 1995). Crisp (1972) originally recommended that if a true breeding, intermediate hybrid between *S. vulgaris* and *S. squalidus* should become established, then it should be given formal taxonomic recognition, in line with this recommendation and the fact that it has

achieved a level of reproductive isolation, York radiate groundsel should warrant recognition as a new species.

Future work

During the execution of practical work and the analysis of results presented in this thesis, several areas in which further work would be interesting and perhaps fruitful, became apparent.

Recently, genomic *in situ* hybridization (GISH) has been used to examine the genomic contribution of parental taxa to hybrids (e.g. in *Milium montianum*, Bennett, Kenton and Bennett, 1992). GISH could be used to examine the relative contributions by *S. vulgaris* and *S. squalidus* to the genome of York radiate groundsel and compare the results to relative contributions present in *S. vulgaris* var. *hibernicus*.

A physical map of the York radiate groundsel genome generated by GISH could also be compared to a genetic map constructed from segregational data of saturating markers (e.g. RAPDs). Several morphological and life history characters, probably derived from *S. squalidus*, have been proposed to be associated with the ray floret locus in different populations of radiate groundsel (Chapter 4; Oxford, Crawford and Pernyes, 1996; Richards, 1975). Recent work on homoploid hybridization in *Helianthus* (Rieseberg, 1995; Rieseberg, Fossen and Desrochers, 1995; Rieseberg *et al.*, 1996), has suggested that chromosomal rearrangements in hybrid progeny may be non-random and groupings of unlinked genes are favoured probably due to their influence on fertility. The study of genomic structure in synthesized and natural hybrid progeny between *S. vulgaris* and *S. squalidus* could offer further insights into the selection for coadapted gene complexes and genomic architecture of hybrid progeny.

As the involvement of unreduced *S. squalidus* gametes has been implicated in the origin of both York radiate groundsel and *S. vulgaris* var. *hibernicus* it would be of interest to assess their production in more detail. Different conditions are known to affect unreduced gamete production (e.g. cold, Bretagnolle and Thompson, 1995). Flow cytometry could be used to assess the frequency with which unreduced gametes are produced in pollen grains (Bretagnolle and Thompson, 1995). Harlan and de Wet (1975) observed that unreduced egg, rather than pollen, production may be more important in the origin of polyploids and this could also be investigated. Finally results from GISH could provide crucial evidence for the involvement of unreduced *S. squalidus* gametes in the origin of York radiate groundsel if the genomic

contribution by *S. squalidus* is shown to be more than that of a reduced gamete (i.e. n=10).

To investigate further the nature of the ethological separation between York radiate groundsel and *S. vulgaris*, more detailed field investigations into pollinator behaviour would be of interest. Of particular importance, would be to assess the levels of inter- and intrataxon outcrossing frequency in ray and disc florets using experimental designs that allow pollinator choice to be expressed (c.f. design used by Irwin, 1990).

It is clear from preliminary work presented in this thesis that York radiate groundsel differs from its two parent taxa in several characters that may affect their respective ecology and niche requirements. Further investigations into the specific ecological requirements and adaptations of York radiate groundsel may be of interest, particularly in respect to those ecological characteristics that it may have inherited from either parent or those that it may have acquired due to its origin or in response to its unique niche.

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Appendix 1.
Isozyme recipes

Lithium Borate system

Used to resolve AAT, EST, GDH isozyme systems;
Electrode buffer; 11.9 g/L ortho-boric acid, 1.2 g/L lithium hydroxide, to pH 8.3.
Gel buffer; 100 ml electrode buffer, 5.45 g/L Tris pH 8.3, 1.28 g/L anhydrous citric acid made up to 1 L with distilled water.

Aspartate Amino Transferase (AAT)

Top or middle gel slice transferred to AAT staining solution (50 ml 0.1 M Tris HCl pH 8.5, 18 mg α-keto glutaric acid, 65 mg aspartic acid, 250 mg PVP-40T, 50 mg EDTA, 710 mg Na_2HPO_4, 5 mg pyridoxyl-5-phosphate, 200 mg Fast Blue BB salt).

Esterase (αEST and βEST)

Top or middle slice transferred to αEST and/or βEST staining solution (40 ml distilled water, 50 ml sodium di-hydrophosphate, 10 ml di-sodium hydrophosphate, 2 ml 1% (w/v) α-naphthyl acetate for aesterase enzymes and/or 1 ml 1% (w/v) β-naphthyl acetate for besterase enzymes, 125 mg Fast Blue RR salt dissolved in 1 ml acetone).

Glutamate dehydrogenase (GDH)

Bottom slice transferred to GDH staining solution (50 ml 0.1 M Tris HCl pH 7.5, 210 mg L-glutamic acid, 0.5 ml 2% (w/v) NAD, 0.5 ml 2% (w/v) MTT, 0.5 ml 1% (w/v) PMS, 0.5 ml (w/v) ATP).

Tris Citrate system

Used to resolve ACO, ACP and IDH isozyme systems;
Electrode buffer; 16.35 g/L Tris, 6.1 g/L monohydrate citric acid to pH 8.0.
Gel buffer; 67 ml electrode buffer, 1.09 g/L Tris, 0.63 g/L monohydrate citric acid, made up to 1 L with distilled water.

Aconitase (ACO)

Top or middle slice transferred to ACO staining solution (45 ml 0.2 M Tris HCl pH 8.0, 1 ml cis-aconitic acid, 2.5 ml 10% (w/v) $MgCl_2$, 0.5 ml 2% (w/v) NADP, 0.5 ml 2% (w/v) MTT, 0.5 ml 1% (w/v) PMS, 300 ml IDH).

Acid Phosphotase (ACP)

Top slice pre-soaked in 50 ml 0.4 M Acetate pH 5.0 for 20 minutes at 4° C. The transferred to ACP staining solution (50 ml 0.2 M acetate pH 5.0, 0.5 ml 1% (w/v) Na a-naphthyl acid phosphate, 1 ml 10% (w/v) $MgCl_2$ $6.H_2O$ and lastly add 40 mg Fast Garnet GBC).

Isocitrate Dehydrogenase (IDH)

Top or middle slice transferred to IDH staining solution (50 ml 0.1 M Tris HCl pH 7.5, 100 mg glucose-6-phosphate, 0.5 ml 2% (w/v) NADP, 0.75 ml 2% (w/v) MTT, 0.5 ml 1% (w/v) PMS, 1 ml 10% Mg Cl_2).